Bull. Pharm. Sci., Assiut University, Vol. 48, Issue 2, 2025, pp. 969-986.

Bulletin of Pharmaceutical Sciences
Assiut University
Website: http://bpsa.journals.ekb.eg/

POTENT ANTI-INFLAMMATORY ACTIVITY OF AFRICAN DAISY RICH IN POLYPHENOLS AND QUINIC ACID DERIVATIVES ON CARRAGEENAN-INDUCED RAT PAW EDEMA

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The current study aims to investigate the in vivo anti-inflammatory and in vitro antioxidant activities of the ethyl acetate fraction of Dimorphotheca ecklonis DC (Compositae), along with its phytochemical composition and safety in gastric tissue. Wistar rats were divided into seven groups to analyze histopathological changes in the paw and gastric tissues treated with extracts of D. ecklonis (African daisy), compared to both positive and negative controls. The thickness of the dermal layers across all groups was measured using ImageJ software. High-Performance Liquid Chromatography (HPLC) analysis and metabolite isolation techniques were employed to identify the phytochemical constituents of the ethyl acetate fraction. Additionally, in vitro antioxidant activity and antihistamine release were evaluated using DPPH and MTT assays.

The ethyl acetate fraction of African daisy demonstrated significant anti-inflammatory activity comparable to that of indomethacin. No significant pathological changes were observed in the gastric tissues of the rats treated with D. ecklonis ethyl acetate extract. Thirteen polyphenols of various classes were detected in the ethyl acetate fraction, including kaempferol, luteolin, apigenin, chlorogenic acid, and 4-caffeoyl-5-feruloyl quinic acid methyl ester. The high phenolic and flavonoid content contributed to its potent antioxidant, antihistamine, and anti-inflammatory activities.

Our study concluded that the ethyl acetate fraction of D. ecklonis exhibits significant anti-inflammatory, antioxidant, and antihistamine activities in both in vivo and in vitro assays. These potent effects are attributed to the synergistic interactions of phenolics and flavonoids, such as kaempferol, luteolin, apigenin, chlorogenic acid, and 4-caffeoyl-5-feruloyl quinic acid methyl ester

Keyword: *Anti-inflammatory, Dimorphotheca. ecklonis, Flavonoids, Quinic acid derivatives, Carrageenan induced paw edema*

INTRODUCTION

Edema occurs when there is an imbalance in water exchange between blood vessels and interstitial spaces. The type of edema commonly observed in the feet and legs, known as peripheral edema, is primarily caused by venous obstruction and increased permeability of blood vessels[1]. This condition can also arise from inflammation, which facilitates the movement of fluid across capillary walls. An acute inflammatory response is characterized by increased vascular leakage and cellular infiltration, resulting in fluid extravasation,

Received : 5/3/2025 & Accepted : 6/7/2025

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including plasma proteins, and the accumulation of white blood cells at the inflammation site[1]. Various mediators are involved in inflammation; for instance, histamine, prostaglandins, and serotonin contribute to increased vascular permeability. Another key mediator in acute inflammation is nitric oxide (NO), produced by nitric oxide synthase (NOS) in response to various pathological conditions[1]. Numerous studies have shown that the pro-inflammatory proteins COX-2 and iNOS play critical roles in the pathophysiology of paw edema, primarily due to their significant production of nitric oxide (NO) and prostaglandin E synthase 2 (PGE2). NO acts as a powerful vasodilator, and its role in inflammatory responses may be linked to its ability to enhance vascular permeability and promote edema formation by altering local blood flow[1,2].

Several synthetic drugs, including NSAIDs such as aspirin, diclofenac, ibuprofen, nimesulide, rofecoxib, and mephenamic acid, as well as corticosteroids, have been commonly used to treat inflammatory disorders. However, due to their potential side effects and serious adverse reactions, they are now regarded with caution and considered unsafe for human use[3]. The severe side effects associated with these synthetic drugs, particularly when used over long periods, include peptic ulcers, hypersensitivity, hyperglycemia, osteoporosis, kidney failure, hepatic failure, and immunodeficiency[3].

In recent decades, herbal products with anti-inflammatory activity have become particularly appealing due to their lower risk of side effects compared to synthetic medications[3]. Various species within the Compositae family have been studied for their anti-inflammatory properties[4]. While many Compositae (Asteraceae) species are recognized for their medicinal benefits, some stand out more than others, especially regarding the patents and products available in the market[4]. Notable anti-inflammatory species within the Compositae family that are traditionally used, listed in pharmacopeias, and commercially available include *Calendula officinalis*, *Matricaria chamomilla*, and *Arnica montana*[4].

Dimorphotheca ecklonis DC. (African daisy) is a species within the genus *Osteospermum* (Family Compositae), formerly classified as *Dimorphotheca*, and is indigenous to South Africa[5]. This sub-shrub is known for its rapid growth and abundant flowering, and it has been distributed worldwide due to its ornamental value[5]. Extracts from the flowers and aerial parts of *D. ecklonis* have shown promising inhibition of rat paw edema compared to indomethacin[6]. Additionally, these extracts exhibit potent in vivo antipyretic, analgesic, and hepatoprotective activities. Five anti-inflammatory phenolic compounds have been isolated from *D. ecklonis* extracts: 3,5-Dicaffeoylquinic acid, neochlorogenic acid, caffeic acid, mearnsitin-3-O-β-D-glucoside, and isoquercitrin. The isolated polyphenols also demonstrated significant inhibition of paw edema, along with antipyretic, analgesic, and hepatoprotective effects[6]. Therefore, further histopathological research on the anti-inflammatory activity of *D. ecklonis* extracts, combined with phytochemical metabolite fingerprinting, and is highly warranted.

The Compositae family, which includes the species selected for our study, holds significant economic value in pharmaceuticals, food, and cosmetics, in addition to serving as ornamental plants[4,7]. This family also comprises species with potential applications in phytomedicine. Beyond their economic benefits, many species within this family have garnered interest for their potential to treat various diseases[4]. Numerous plants have been traditionally utilized for their healing properties, including arnica (*Arnica montana*), globe artichoke (*Cynara scolymus*), guaco (*Mikania glomerata* var. glomerata), and macela (*Achyrocline satureioides* Lam. DC.). These plants are commonly recognized for their anti-inflammatory effects, support of liver health, and treatment of respiratory conditions[4,7].

Several in vitro and in vivo studies have demonstrated the anti-inflammatory activity of extracts from Compositae species. The most commonly reported in vitro methods for assessing anti-inflammatory effects include the inhibition of cytokine (NO) release from macrophages, as well as the inhibition of cyclooxygenase, prostaglandins, and NF-kB. For instance, the essential oil extracted from the flowers of *Cotula anthemoides*, a wild Egyptian plant, showed promising inhibition of

cyclooxygenase-2, pro-inflammatory cytokines, and tumor necrosis factor-α in RAW 264.7 macrophage cells[8]. Extracts from certain Arnica species, particularly those rich in helenanolide-type sesquiterpene lactones such as *A. chamissonis* and *A. montana*, exhibited potent inhibition of the transcription factor NF-kappa B[9]. The ethyl acetate fraction and ethanol extract of *Vernonia condensata*, which are rich in phenolics and flavonoids like 1,5-diphenoylquinic acid, apigenin, luteolin, and chlorogenic acid, demonstrated inhibition of NO release, as well as reduced production of IL-6 and TNF-α[10]. Additionally, the extract of *Centaurea athoa* at a dose of 50 mg/kg exhibited anti-inflammatory activity, as evidenced by the carrageenan-induced paw edema test in rats[11]. Extracts from *Mikania* species, including *M. cordata* and *M. lindleyana*, also attenuated carrageenan-induced rat paw edema in a dose-dependent manner at doses ranging from 100 to 800 mg/kg[4]. Lastly, the methanol extract of the aerial parts of *Centaurea iberica* demonstrated dose-dependent anti-inflammatory activity in acetic acid-induced rat edema[12].

Several secondary metabolites from various classes responsible for the anti-inflammatory activity of Compositae species have been isolated. The in vivo and in vitro anti-inflammatory effects of these compounds have been well documented. For instance, thirty flavonoids from the flavanone, flavone, and flavonol classes, isolated from Compositae species, were tested for their anti-inflammatory activity using carrageenan-induced paw edema and cotton pellet-induced rat granuloma methods[13]. These flavonoids were found to inhibit granuloma formation at a specific dose of 75 mg/kg administered intraperitoneally, particularly when the compound structure included a catechol B ring[13].Caffeoylquinic acid isolated from *G. stramineum* flower extract, including 4-O-caffeoylquinic, 3,5-di-O-caffeoylquinic, and 4,5-di-O-caffeoylquinic acids, demonstrated promising anti-inflammatory activity on human macrophages by inhibiting superoxide anion production and monocyte migration[14]. Additionally, glycosides of quercetin (rutinoside, glucoside, and galactoside) isolated from the same plant exhibited significant attenuation of carrageenan-induced paw edema and cotton pellet granuloma exudation[14]. Artemisinin and parthenolide, sesquiterpene lactones isolated from various Compositae species, are commercially available as anti-inflammatory agents[15]. Moreover, cynaropicrin, arglabin, lactucin, helenalin, costunolide, inuviscolide, parthenolide, tomentosin, and thapsigargin are used as positive controls in several in vivo studies[15]. Finally, eleven triterpene alcohols, including 24-methyl-enecycloartanol, heliaol, cycloartenol, taraxasterol, lupeol, psi-taraxasterol, alpha-amyrin, beta-amyrin, taraxerol, tirucalla-7,24-dienol, and dammaradienol, isolated from fifteen Compositae flowers, demonstrated anti-inflammatory activity against 12-O-tetradecanoylphorbol-13-acetate-induced ear inflammation in mice[16].

In our study, we screened the methanol extracts of the aerial parts of three Compositae species *Senecio cruentus*, *Dimorphotheca ecklonis*, and *Arctotis aurantiaca*, for their ability to inhibit histamine release in U937 human monocytes. The active extracts and fractions were subsequently investigated for their antioxidant and anti-inflammatory activities using a carrageenan-induced paw edema model. Additionally, we assessed their phenolic and flavonoid content and conducted chemical characterization on the most active fraction.

MATERIAL AND METHODS

Solvents and chemicals

The solvents used in this study for extraction and fractionation; methanol, hexane, chloroform, and ethyl acetate were obtained from Alfa Chemicals in Cairo, Egypt. Methanol and acetonitrile, used for elution in the HPLC experiments, were HPLC-grade solvents purchased from Thermo Fisher Scientific (Pittsburgh, USA). The chemicals used to determine phenolic and flavonoid contents, as well as antioxidant activity, included Folin-Ciocalteu's phenol reagent, gallic acid, quercetin, $AlCl_3$, DPPH, and trolox, all sourced from Sigma-Aldrich (Burlington, MA, USA). Hematoxylin and eosin kits for the histopathological study were purchased from Abcam (Cambridge, UK). TLC plates, RP-TLC plates, normal column silica gel plates, reversed-phase C18 silica gel plates, and

Sephadex LH20 were sourced from Merck (Darmstadt, Germany) and used for the compound isolation process. Diclofenac and indomethacin, NSAID reference compounds obtained from October Pharmaco (Cairo, Egypt), served as positive controls for antihistamine and anti-inflammatory studies.

Plant materials

The aerial parts of *Dimorphotheca ecklonis* (African daisy), *Senecio cruentus*, and *Arctotis aurantiaca* were collected from the Plant Garden of Horticulture and Aromatics at the Faculty of Agriculture, Minia University, Egypt, in April 2020. Prior to the collection of the plant materials, formal permission was obtained from the director of the Plant Garden. The selected plants were identified by Dr. Mahmoud Abdelhady Hassan, a professor of aromatic plants and horticulture. Voucher specimens were deposited in the herbarium of the Pharmacognosy Department at the Faculty of Pharmacy, Assiut University, Egypt, with the following voucher numbers: 2221984 (*D. ecklonis*), 2221985 (*A. aurantiaca*), and 2221983 (*S. cruentus*).

Extraction and fractionation

Two kilograms of dried powdered aerial parts were extracted with 70% methanol (4000 mL) at room temperature (25 °C) under shaking for 72 hours. This extraction process was repeated three times until exhaustion. The methanol extract from each plant was concentrated under reduced pressure using a rotary evaporator. For fractionation, 200 g of the methanol extracts were suspended in 500 mL of 10% methanol using a 2L separating funnel. The aqueous extract of each plant was further fractionated using hexane, dichloromethane, and ethyl acetate solvents (1000 mL each, three times per solvent). The hexane, dichloromethane, and ethyl acetate fractions were collected, concentrated, and stored at -20 °C for subsequent chemical and biological studies.

In vitro antihistamine release

The total methanol extracts of the three plants, along with the various fractions (hexane, dichloromethane, ethyl acetate, and aqueous) of *Dimorphotheca ecklonis,* were tested for their ability to inhibit histamine release in vitro. For this assay, 96-well plates were cultured with U937 human monocytes obtained from ATCC (Manassas). The enzyme immunoassay (EIA) kit from SPI-Bio (France) was used, following established protocols[17]. Diclofenac, sourced from October Pharmaco (Cairo, Egypt), served as a positive control. The inhibition of histamine release was calculated using the following equation:

$$\text{Inhibitory activity (\%)} = (1 - A_s/A_c) \times 100.$$

Where, A_c is the control absorbance and A_s is the absorbance of tested extract. To calculate the IC_{50} values, triplicate measurements for each concentration were performed using a UV2000 spectrophotometer (Ray Wild Limited Company, L569 Gottingen, Germany) at 405 nm, and the results were expressed as the mean ± SD.

Total phenolic contents

The phenolic content of the ethyl acetate fraction of *Dimorphotheca ecklonis* was determined using a previously published method. Briefly, a serial dilution of gallic acid (the standard compound) was performed in distilled water, resulting in a concentration range of 100 to 10 ppm[18]. To the ethyl acetate fraction and the prepared gallic acid solutions (1:1), 0.2 mL of Folin-Ciocalteu's reagent was added. After 5 minutes, 1 mL of sodium carbonate and 1.6 mL of distilled water were incorporated into the mixtures. All standard and tested solutions were kept in the dark for 30 minutes. The samples were then centrifuged and measured in triplicate using a UV2000 spectrophotometer (Ray Wild Limited Company, L569 Göttingen, Germany) at 750 nm. A gallic acid standard curve was prepared **(Fig S1)**, and the phenolic content of the ethyl acetate fraction of *D. ecklonis* was calculated as the mean of triplicate measurements ± SD, expressed in mg/g dry weight GAE (Gallic Acid Equivalent).

Total flavonoid contents

The flavonoid content of the ethyl acetate fraction of *Dimorphotheca ecklonis* was determined using a previously published method[19]. Briefly, a serial dilution of quercetin (the standard compound) was performed in methanol, resulting in a concentration range of 100 to 10 ppm. A 2% $AlCl_3$ reagent (0.6 mL)

was added to all standard solutions and the tested ethyl acetate extract of *D. ecklonis* (1:1). The mixtures were then left to stand for 60 minutes at room temperature. Subsequently, the samples were measured in triplicate using a UV2000 spectrophotometer (Ray Wild Limited Company, L569 Göttingen, Germany) at 415 nm. A quercetin standard curve was prepared **(Fig S2)**, and the flavonoid content of the ethyl acetate fraction of D. ecklonis was calculated as the mean of the triplicate measurements ± SD, expressed in mg/g dry weight QE (Quercetin Equivalent).

Antioxidant DPPH assay

The affinity of the methanol extract and ethyl acetate fraction of *Dimorphotheca ecklonis* for scavenging free radicals was measured using the DPPH method[20]. Trolox reference samples (60 - 1000 µg/mL in ethanol) and tested extract samples (60 - 1000 µg/mL in ethanol) were prepared, along with a DPPH (2,2-diphenyl-1-picrylhydrazyl) stock solution at 200 µM. Three hundred microliters of DPPH were added to 300 µL of each control or tested sample and allowed to react in the dark at room temperature for 30 minutes. The absorbance of the tested and control samples was measured using a UV2000 spectrophotometer (Ray Wild Limited Company, L569 Göttingen, Germany) at 517 nm. The percentage of antioxidant activity was calculated using the following equation:

$$SA\% = (A_0 - A_s/A_0) \times 100.$$

where A_0 is the absorbance of DPPH solution in ethanol and A_s is the absorbance of tested extract and DPPH.

HPLC analysis of D. ecklonis ethyl acetate fraction

Detection of phenolic compounds

A 25 µL injection of the ethyl acetate fraction of *Dimorphotheca ecklonis* was analyzed at 280 nm using an Agilent 1100 HPLC system equipped with a UV/Vis detector and operated with Agilent ChemStation software. The separation of phenolic compounds was achieved using an Agilent 959961-902 ZORBAX RR Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 µm) in a gradient elution solvent system. Solvent A consisted of acetic acid in water (0.01%), while solvent B was 100% methanol. The experiment commenced with 100% acetic acid in water for 3 minutes, followed by a transition to 50% methanol for 5 minutes, then 80% methanol for the next 2 minutes, and finally returning to 50% methanol for an additional 5 minutes. Phenolic compounds were identified using authentic samples.

Detection of flavonoids compounds

A 25 µL injection of the ethyl acetate fraction of *Dimorphotheca ecklonis* was analyzed at 320 nm using an Agilent 1100 HPLC system equipped with a UV/Vis detector and operated with Agilent ChemStation software. The separation of flavonoids was achieved using an Agilent 959961-902 ZORBAX RR Eclipse Plus C18 column (100 mm × 4.6 mm, 3.5 µm) in an isocratic elution solvent system, where solvent A consisted of 1% formic acid in water and solvent B was 100% acetonitrile. The isocratic system maintained a composition of 30% solvent A and 70% solvent B for 15 minutes. Flavonoids were identified using authentic samples.

Isolation of quinic acid derivative and phenolic acid

The ethyl acetate fraction of *Dimorphotheca ecklonis* was mixed with silica gel in a 1:3 ratio, then dried and powdered. This mixture was subjected to Vacuum Liquid Chromatography (VLC) using a column packed with a thin layer of silica gel over a thicker layer for thin-layer chromatography (TLC). Negative pressure was applied with an electric pump to accelerate the elution process. The column was initially eluted with dichloromethane (DCM), followed by a gradient of DCM and methanol (MeOH) until reaching 100% MeOH. Similar fractions were combined based on their TLC profiles and concentrated under reduced pressure, resulting in six fractions. These fractions were further concentrated, dried, and monitored using TLC. Two compounds (7, 8) were isolated from different sub-fractions using multiple open columns containing normal silica gel, Sephadex LH-20, and RP-18. The isolated compounds were stored at -20 °C until NMR spectroscopic analyses were conducted (Bruker, Billerica, Massachusetts, USA).

Animals and experimental design

Thirty-five male Wistar rats weighing 180 ± 20 grams were used to evaluate the in vivo anti-inflammatory efficacy of the methanol and ethyl acetate extracts of *Dimorphotheca ecklonis* at oral doses of 100 and 400 mg/kg. The albino rats were obtained from the Animal Housing Center at the Faculty of Medicine, Sohag University, and were kept under standard conditions, with access to food and water, at the pharmacology department's animal lab for 7 days prior to the experiment. This study was conducted following international regulations and guidelines, with the experimental protocol approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine, Sohag University (Approved No. 12-7-7/2024-01).

The rats were divided into seven groups: a control group, four treated groups, a carrageenan-induced disease group, and an indomethacin-treated group. Carrageenan was chosen to induce paw edema in the rats, following previously reported methods[21-24]. The experimental animals received an injection of 100 µL of freshly prepared 1% carrageenan in saline. The rats in the treatment groups were administered either the ethyl acetate fraction or the methanol extract of *D. ecklonis*, freshly prepared in 1% carboxymethylcellulose (CMC), at doses of 100 and 400 mg/kg, 60 minutes prior to the carrageenan injection. The positive control group received indomethacin orally at a dose of 10 mg/kg[25], while the control group received 0.3 mL of saline 1 hour before the carrageenan injection. The doses used in this experiment were selected based on previously reported ranges for other Compositae species, including *D. ecklonis* (50 - 800 mg/kg)[6-7]. Additionally, no signs of acute toxicity or mortality were observed in rats receiving the *D. ecklonis* extracts and fractions at doses up to 1000 mg/kg. The LD_{50} (lethal dose 50%) values for the aqueous and alcoholic extracts, as well as the ethyl acetate fraction, were previously reported as $LD_{50} \geq 6$ g/kg[6], confirming the safety of the doses used in this study.

Histopathological examination

The paw and stomach tissues of the rats from all groups were dissected into sections that were 5 µm thick and embedded in paraffin wax. These sections were subsequently stained with hematoxylin and eosin according to established protocols[26]. The architecture and inflammatory cellular response of the paw and stomach mucosa were examined using an Olympus CX 41RF light microscope (Olympus Corporation, Tokyo, Japan).

Statistical analysis

The data from the histopathological and histamine release inhibition studies were summarized in figures created using GraphPad Prism software (version 6.0, GraphPad Software, California, USA). Means ± standard deviations (S.D.) were reported for each group of five rats in the anti-inflammatory assay. Similarly, means ± standard deviations for each concentration of plant extract, based on triplicate measurements, were provided for all in vitro experimental results related to histamine release inhibition. Data from different groups were compared using one-way analysis of variance (ANOVA), with post hoc Tukey-Kramer multiple comparisons applied. Differences were considered significant at $P < 0.05$ (*), highly significant at $P < 0.01$ (**), and very highly significant at $P < 0.001$ (***).

RESULTS AND DISCUSSION

Inhibition of histamine release

Three species from the Compositae family cultivated in Egypt were selected to investigate the effects of their methanol extracts on histamine release inhibition in U937 human monocytes. These species include *Dimorphotheca ecklonis* (African daisy), *Senecio cruentus*, and *Adenostyles aurantiaca*. The antihistamine release activity of the methanol extracts from these three species is summarized in **Table 1** and illustrated in **Fig. 1**.

The active methanol extract of *D. ecklonis* was chosen for further investigation of histamine release inhibition through its fractions, as detailed in **Fig. 1** and **Table 2**.

Table 1: Histamine release % inhibition of the aerial parts methanol extracts of *D. ecklonis* (African daisy), *A. aurantiaca*, and *C. cruenta*.

Sample conc. (µg/mL)	Histamine release inhibition (%)			
	D. ecklonis	***A. aurantiaca***	***C. cruenta***	**Diclofenac**
1000	72.65 ± 0.58**	0	64.31 ± 1.2*	86.34**
500	70.14 ± 2.1**	0	58.32 ± 1.2*	79.25**
250	65.46 ± 1.5**	0	53.18 ± 0.58*	68.32**
125	61.32 ± 0.63**	0	46.64 ± 0.63*	61.35**
62.5	56.32 ± 0.58**	0	29.84 ± 0.7*2	57.32**
31.25	52.22 ± 1.2**	0	17.61± 1.3*	54.32**
15.63	28.98 ± 1.5*	0	10.35 ± 1.5*	49.25**
7.81	11.43 ± 1.5*	0	0	36.32**
IC_{50}	29.7	-	189.2	17.9

$P \leq 0.05$ (*) and $P \leq 0.01$ (**).

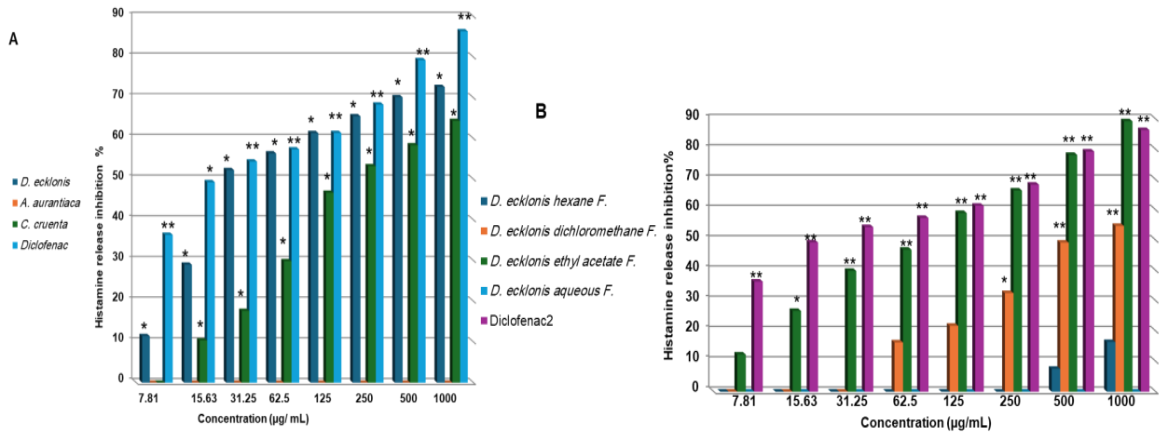

Fig. 1: Inhibition of histamine release (A & B). A: Percentage of inhibition in the release of the Compositae species *D. ecklonis, A. aurantiaca, and C. cruenta*. **B:** Percentage of inhibition in the release of African daisy (D. ecklonis) hexane, dichloromethane, ethyl acetate, and aqueous fractions. $P \leq 0.05$ (*) and $P \leq 0.01$ (**).

Determination of the antioxidant properties of African daisy extracts, as well as the total phenolic, total flavonoid contents of African daisy ethyl acetae fraction

The total methanol extract and the ethyl acetate fraction of *Dimorphotheca ecklonis* (African daisy) exhibited the most potent activity against histamine release **(Fig. 1 and Tables 1 & 2)** and were selected for further study on their capacity to scavenge DPPH, using trolox as a reference compound. The ethyl acetate fraction demonstrated activity eight times greater than that of the total methanol extract, with IC_{50} values of 102.5 ± 1.021 µg/mL and 851.4 ± 1.034 µg/mL, respectively **(Fig. S3)**.

Subsequently, the total phenolic content of the ethyl acetate fraction was determined using the Folin-Ciocalteu assay[18]. The fraction contained 6.97 ± 0.26 mg/g dry weight of Gallic acid equivalent, as shown in **Fig. S3**. Similarly, the flavonoid content of the ethyl acetate fraction was calculated to be 14.36 ± 0.47 mg/g dry weight of Quercetin Equivalent (QE) using the aluminum chloride colorimetric assay[19].

Table 2: Histamine release % inhibition of *D. ecklonis* fractions.

Sample conc. (µg/mL)	Histamine release inhibition (%)				
	***n*-Hexane fraction**	**Dichloro-methane fraction**	**Ethyl acetate fraction**	**Aqueous fraction**	**Diclofenac**
1000	16.35 ± 0.72	54.69 ± 1.6**	89.25 ± 0.58**	0	86.34**
500	7.52 ± 1.2	49.18 ± 2.2**	78.14 ± 1.3**	0	79.25**
250	0	32.52 ± 0.85*	66.38 ± 0.58**	0	68.32**
125	0	21.73 ± 1.4	58.96 ± 2.3**	0	61.35**
62.5	0	16.24 ± 2.1	46.85 ± 0.93**	0	57.32**
31.25	0	0	39.85 ± 1.4**	0	54.32**
15.63	0	0	26.63 ± 1.9*	0	49.25**
7.81	0	0	12.25 ± 2.1	0	36.32**
IC_{50}	>1000	574.4	98.0	-	17.9

$P \leq 0.05$ (*) and $P \leq 0.01$ (**).

HPLC analysis of the African daisy ethyl acetate extract

The ethyl acetate fraction of *Dimorphotheca ecklonis* was subjected to HPLC-UV analysis in two distinct experiments. An isocratic elution was employed to separate the flavonoids at 320 nm, while gradient elution was used for the separation of simple phenolic compounds at 280 nm. All separated compounds, detailed in **Table 3** and illustrated in **Fig. 2**, were identified using authentic samples. The HPLC chromatograms revealed the separation of six phenolic compounds and five flavonoids **(Fig. S4)**.

Table 3: List of phenolic compounds and flavonoids detected in *D. ecklonis* ethyl acetate fraction by HPLC.

Peaks no.	Rt (Retention time in min)	Compounds	Concentration (µg/mL)
Phenolics			
1	4.0	Gallic acid	5.61
2	5.0	Pyrogallol	13.74
3	6.89	Cinnamic acid	5.07
4	7.95	Chlorogenic acid	4.80
5	10	P-hydroxyl benzoic acid	4.55
6	14.3	Sinapic acid	5.33
Flavonoids			
7	8.1	Kaempferol	10.36
8	9.0	Luteolin	5.12
9	10.0	Apigenin	4.78
10	12.0	Catechin	2.66
11	14.9	Avicularin	1.47

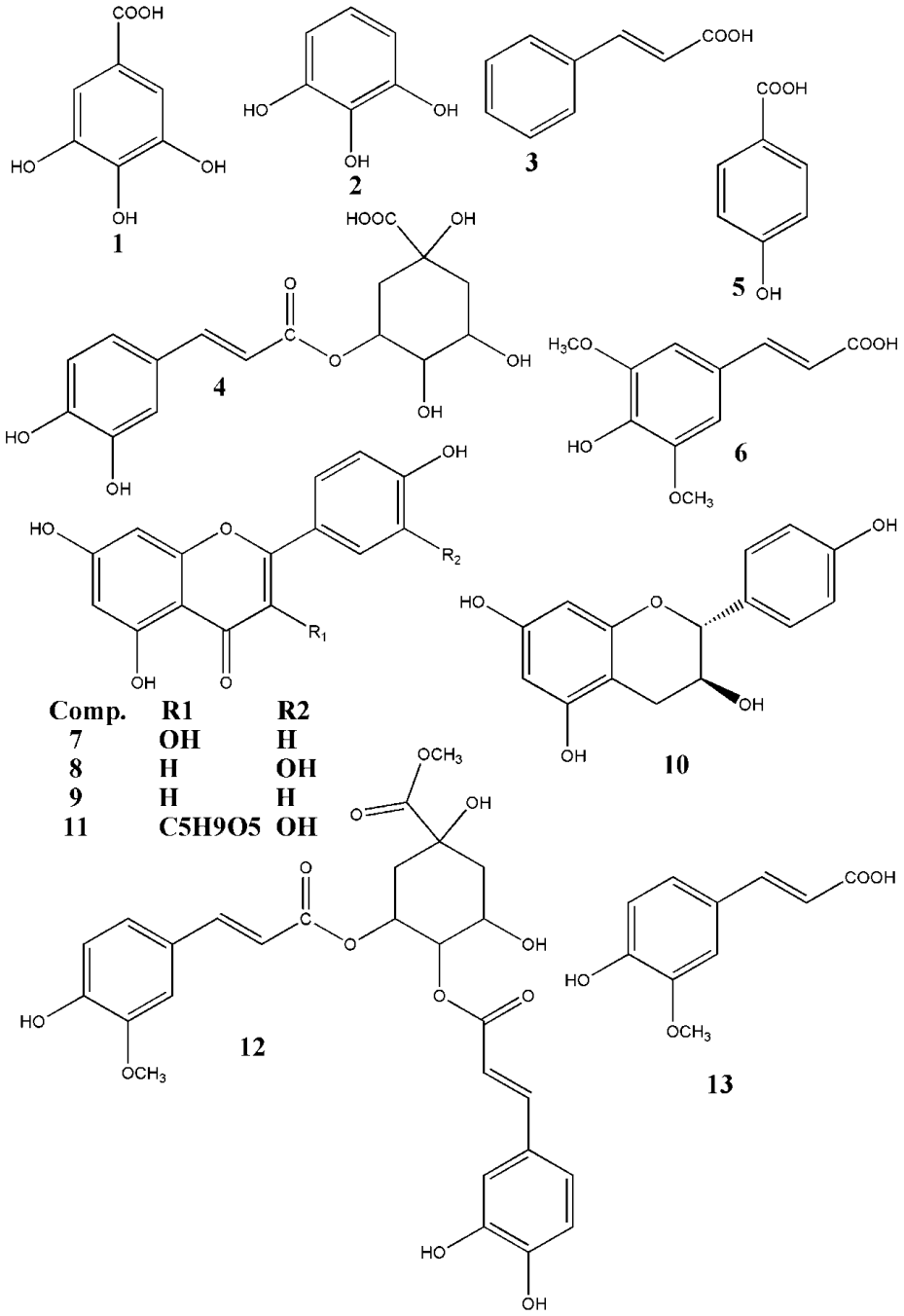

Fig. 2: Structure of compounds identified in the African daisy ethyl acetate fraction.

Compounds isolated from African daisy ethyl acetate

4-caffoeyl -5-feruloyl quinic acid methyl ester (12) (10 mg), yellow amorphous powder. ^{1}H-NMR (Methanol-d_4, 400 MHz) **(Fig. S5)**: δ 7.82 (1H, d, *J* = 1.9, H2'), δ 7.61 (1H, d, *J* = 15.9, H7'), 7.20 (1H, d, *J* = 1.8, H2"), δ 7.14 (1H, dd, *J* = 8.2, 1.8, H6"), δ 7.10 (1H, dd, *J* = 8.2, 1.9, H6'), δ 6.88 (1H, d, *J* = 13, H7"), δ 6.83 (1H, d, *J* = 8.2, H5"), δ 6.81 (1H, d, *J* = 8.2, H5'), δ 6.28 (1H, d, *J* = 15.9, H8'), δ 5.76 (1H, d, *J* = 13, H8"), δ 5.29 (1H, m, H5), δ 4.13 (1H, m, H3), δ 3.85 (3H, s, OCH_3), δ 3.68 (1H, m, H4), δ 3.7 (3H, s, OCH_3), δ 2.21 (2H, m, H2β, H2α), δ 2.03 (2H, m, H6β, H6α).^{13}C-NMR (Methanol-d_4, 100 MHz) **(Fig. S6)**: δ 175.3 (C7), δ 168.2 (C9'), δ 167.5 (C9"), δ 150 (C4'), δ 149.3 (C4"), δ 148.3 (C3"), δ 148.0 (C7'), δ 147.3 (C7"), δ 145.6 (C3'), δ 127.9 (C1"), δ 127.5 (C1'), δ 126.8 (C6"), δ124.3 (C6'), δ 116.5 (C5'), δ 116 (C5"), δ 115.9 (C8"), δ 115.5 (C2"), δ 115 (C8'), δ 114.9 (C2'), δ 75.8 (C1), δ 72.0 (C4), δ 71.7 (C5), 70.3 (C3), 56.3 (OCH3), δ 52.9 (OCH3), δ 37.3 (C6), δ 37.28

(C2). The identification of compound **12** was confirmed by DEPT-135 as shown in **Figs. S7** in addition to a comparison the ^{1}H and ^{13}C-NMR data with those previously published results[27]**.**

Ferulic acid (13) (5 mg), yellow amorphous powder. ^{1}H-NMR (Methanol-*d4*, 400 MHz) (**Fig. S8)**: δ 7.53 (1H, d, *J* = 15.9, H7), δ 7.06 (1H, d, *J* = 1.9, H2), 6.96 (1H, dd, *J* = 8.2, 1.9, H6), δ 6.80 (1H, dd, *J* = 8.2, H5), δ 6.23 (1H, d, *J* = 15.9, H8), δ 3.7 (3H, s, OCH3). The ^{1}H-NMR data of compound (**13**) were compared with those previously published results to confirm identification[28].

Effects of African daisy ethyl acetate fraction and methanol extract on paw edema formation

To evaluate the anti-inflammatory effects of extracts with significant histamine release inhibition from three Compositae species in U937 human monocytes, the methanol extract and ethyl acetate fraction of *Dimorphotheca ecklonis* (African daisy) were selected for efficacy testing using the paw edema model, a widely employed method for screening the anti-inflammatory effects of drugs[29-32]. Following a 5-hour subcutaneous injection of 1% carrageenan in all animal groups except the control, a significant difference in paw edema size and redness was observed, as shown in **Fig. 3**. The differences in paw edema volume among the animal groups were quantified by measuring the percentage of edema inhibition at 1, 3, and 5 hours, as illustrated in **Fig. 3** and **Table 3**. The percentage of inflammation inhibition was calculated using the following equation:

Percentage inhibition = (WC-WT/WC) × 100,

where WC is the increase in paw thickness of the control group while WT is the increase in paw thickness of treated rats with test extracts.

Histopathological findings on rat paw tissues

The efficacies of the ethyl acetate fraction and methanol extract of *Dimorphotheca ecklonis* (African daisy), compared to indomethacin, are summarized in **Fig. 4**. The paw tissues in the control group appeared normal, showing no signs of inflammation. In contrast, the paw tissues of carrageenan-treated animals exhibited acute inflammation, characterized by edema and neutrophil migration. Conversely, rats treated with the tested extracts, as well as indomethacin, demonstrated a significant reduction in inflammation and regeneration of paw tissues, except for those treated with the methanol extract of African daisy at 100 mg/kg **(Fig. 4)**. Additionally, differences in relative dermal thickness among the treated groups, indomethacin, the control group, and the carrageenan group were quantified using ImageJ software. The quantitative data are presented as mean ± standard deviation (n = 3) **(Fig. 4)**.

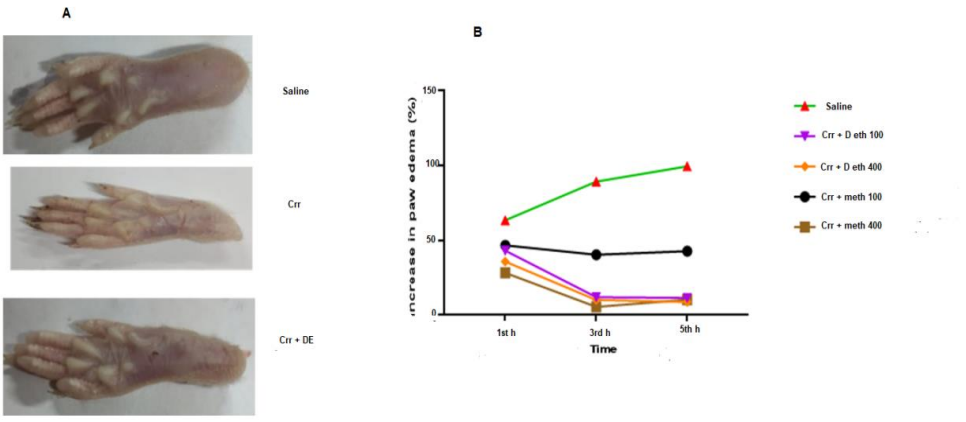

Fig. 3: Effect of African daisy extracts on paw edema (A & B). A: Comparative photographs showing the differences in morphology and size of paw edema between the normal (saline), carrageenan-treated (Carr.), and ethyl acetate-treated (Carr. + DE) groups at 100 mg/kg. **B:** Percentage of increase in paw edema among the four treated groups: ethyl acetate fraction at 100 mg/kg (D eth 100), ethyl acetate fraction at 400 mg/kg (D eth 400), methanol extract at 100 mg/kg (D meth 100), and methanol extract at 400 mg/kg (D meth 400), compared to the indomethacin group (10 mg/kg).

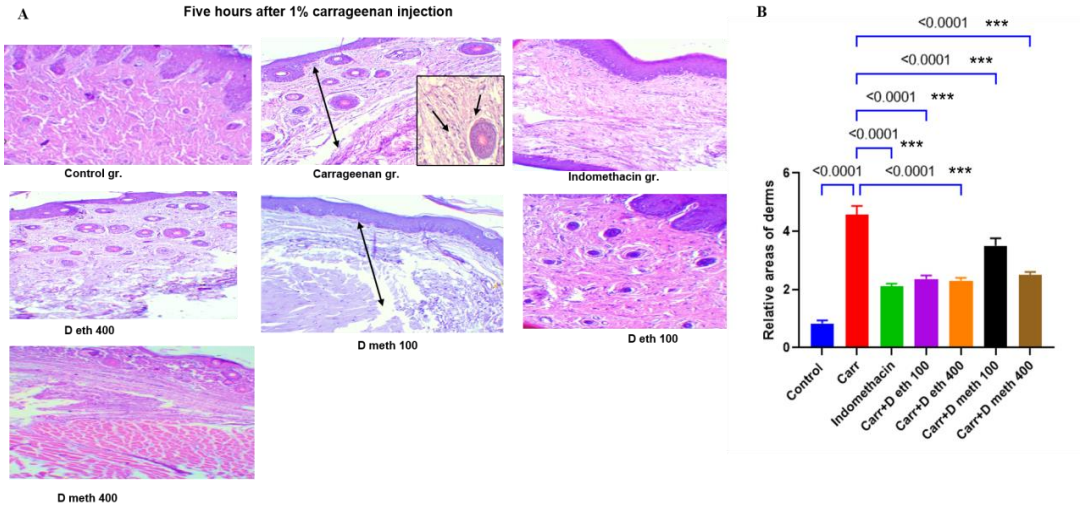

Fig. 4: Histological analysis of paw tissues (A & B). A: Photomicrograph of paw sections stained with H & E. Normal paw tissues are shown in five groups: normal (saline) group, ethyl acetate fraction at 100 mg/kg (D eth 100), ethyl acetate fraction at 400 mg/kg (D eth 400), methanol extract at 400 mg/kg (D meth 400), and the indomethacin group. Inflamed tissue and edema are evident in the carrageenan-treated group (Carr.) and in the methanol extract at 100 mg/kg (D meth 100). **B:** Quantification of the thickness of dermal layers in the control group, carrageenan-treated group (Carr.), ethyl acetate fraction at 100 mg/kg (D eth 100), ethyl acetate fraction at 400 mg/kg (D eth 400), methanol extract at 100 mg/kg (D meth 100), methanol extract at 400 mg/kg (D meth 400), and the indomethacin group using ImageJ software $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***).

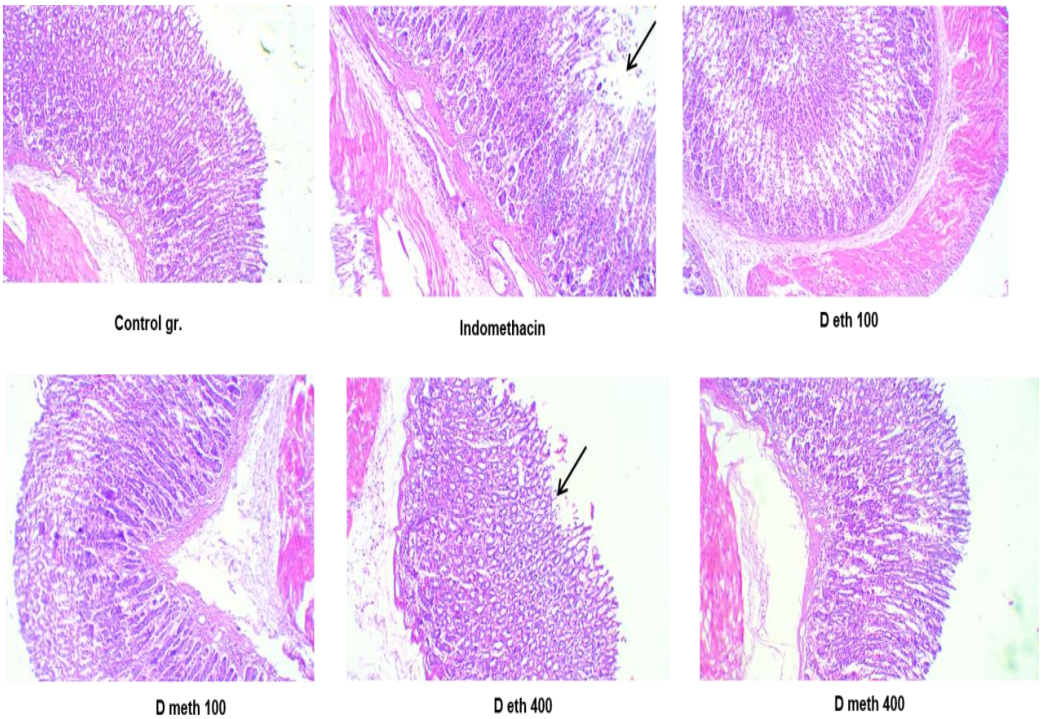

Fig. 5: Histological analysis of gastric tissues. Normal mucosa and submucosa tissues are observed in the normal group, ethyl acetate fraction at 100 mg/kg (D eth 100), methanol extract at 100 mg/kg (D meth 100), and methanol extract at 400 mg/kg (D meth 400). Slight mucosal degeneration is noted in the ethyl acetate fraction at 400 mg/kg (D eth 400). Notable ulcerative regions are observed in the indomethacin-treated group.

Effect of African daisy extracts on rat gastric mucosa

Gastric ulcers are among the most common side effects of certain NSAIDs, including aspirin and indomethacin[33]. In our current study, histopathological examination of the group receiving indomethacin as a reference anti-inflammatory compound revealed a significant degree of degeneration **(Fig. 5)**. Notably, the glandular mucosa exhibited focal ulcerative regions marked by the shedding of the epithelial lining and the accumulation of necrotic tissue. In contrast, rats treated with the tested extracts primarily displayed intact histological structures in both the glandular mucosa and submucosa across the various areas examined **(Fig. 5)**. Only the high dose of the African daisy ethyl acetate extract at 400 mg/kg showed slight mucosal degeneration, which was not statistically significant **(Fig. 5)**.

Discussion

Three Compositae species were screened for their ability to inhibit histamine release in U937 human monocytes, as shown in **Table 1 and Fig. 1**. The methanol extract of *Dimorphotheca ecklonis* (African daisy) exhibited potent activity against histamine release, with an IC50 value of 29.7 ± 0.12 µg/mL. In contrast, the other two species, *S. cruentus* and *A. aurantiaca*, showed weak to no activity, with IC_{50} values of 189.1 ± 0.2 µg/mL and 0 µg/mL, respectively. Bio-guided fractionation of the African daisy methanol extract identified the active ethyl acetate fraction, which had an IC_{50} of 98.0 ± 0.12 µg/mL. Other fractions displayed little to no activity **(Fig. 1 & Table 2).** Both the active antihistamine extracts—the African daisy methanol extract and its ethyl acetate fraction—were selected for further investigation of in vitro antioxidant activity using the DPPH method and in vivo anti-inflammatory activity using carrageenan-induced rat paw edema.

The ethyl acetate fraction exhibited activity eight times greater than that of the total methanol extract, with IC_{50} values of 102.5 ± 1.021 µg/mL and 851.4 ± 1.034 µg/mL, respectively **(Fig. S3)**. Both the ethyl acetate and methanol extracts of *Dimorphotheca ecklonis* demonstrated a dose-dependent ability to neutralize DPPH radicals. As the concentration of the extracts increased, so did their antioxidant activity. According to the literature, the ethyl acetate fraction is rich in flavonoids and phenolic acids, which contribute to its antioxidant properties[34,35].

Fig. 2 illustrates the structures of thirteen different chemical compounds identified through HPLC, including the isolation and structural elucidation of compounds from the ethyl acetate fraction. Eleven compounds from various chemical classes were identified using authentic samples, as detailed in **Table 3**. Among these, five simple phenolic compounds—gallic acid, pyrogallol, cinnamic acid, p-hydroxybenzoic acid, and sinapic acid—along with the quinic acid derivative chlorogenic acid, were detected in the ethyl acetate fraction using a gradient HPLC experiment **(Fig. S4)**. In a subsequent isocratic HPLC experiment, five flavonoids—kaempferol, luteolin, apigenin, avicularin, and catechin—were also identified **(Fig. S4)**.

Additionally, 4-caffeoyl-5-feruloyl quinic acid methyl ester (**12**) and ferulic acid (**13**) were isolated using different chromatographic techniques. Their identification was performed through 1D NMR spectroscopic analysis, including ^{1}H-NMR, ^{13}C-NMR, and DEPT-135, and was confirmed by comparing the data with previously reported results. The ^{1}H-NMR spectral data of compound **12 (Fig. S5)** showed the presence of six aromatic protons with an ABX spin system forming two tri-substituted benzene rings at δH 7.82 ppm (d, J = 1.9 Hz, 1H), 6.83 ppm (d, J = 8.2 Hz, 1H), 7.10 ppm (dd, J = 8.2, 1.7 Hz, 1H), 7.20 ppm (d, J = 1.8 Hz, 1H), 6.83 ppm (d, J = 8.2 Hz, 1H), and 7.14 ppm (dd, J = 8.3, 1.8 Hz, 1H), along with four trans-olefinic protons with an AB system at δH 7.61 ppm (d, J = 15.9 Hz, 1H), 6.28 ppm (d, J = 15.9 Hz, 1H), 6.88 ppm (d, J = 13.0 Hz, 1H), and 5.76 ppm (d, J = 12.9 Hz, 1H), as well as two methoxyl groups at 3.3–3.7 ppm from the feruloyl moiety and quinic acid methyl ester. The protons of quinic acid were consistent with previously reported data for C4 and C5 substituted quinic acid.[27] The ^{13}C-NMR spectral data of compound 12 **(Fig. S6)** showed the presence of three carbonyl groups at 168.2 ppm for C-9’ of the

feruloyl moiety, 167.5 ppm for C-9'' of the caffeoyl moiety, and 175.3 ppm for C-7 of quinic acid methyl ester. It also indicated two methoxyl carbons at 52.9 and 56.3 ppm, and four olefinic carbons at 148.0, 115.0, 147.3, and 115.5 ppm for C-7', C-8', C-7'', and C-8'', respectively. Additionally, aromatic carbons of the feruloyl and caffeoyl moieties were found in the range of 111-150 ppm, and quinic acid carbons were in the range of 70-75 ppm. The carbon types in the structure of compound 12 are presented in the DEPT-135 analysis **(Fig. S7)** as follows: CH and CH_3 groups are upward, CH_2 is downward, with the disappearance of quaternary carbon groups. Compound **12** was identified as 4-caffeoyl-5-feruloyl quinic acid methyl ester[27]. The ^{1}H-NMR data of compound 13 **(Fig. S7)** showed the presence of three aromatic protons at δ 7.06 ppm (1H, d, 1.9 Hz), 6.80 ppm (1H, d, 8.2 Hz), and 6.96 ppm (1H, dd, 8.2, 1.9 Hz), confirming the presence of a tri-substituted aromatic ring in an ABX system. Additionally, two olefinic protons in trans configuration were observed at 6.23 ppm (1H, d, 15.9 Hz) and 7.54 ppm (1H, d, 15.9 Hz), along with singlet methoxyl protons at δ 3.7 ppm. Compound **13** was identified as ferulic acid[28].

The quantification of phenolic content and flavonoids in the ethyl acetate fraction is reported for the first time in this study. The total phenolic content of the African daisy ethyl acetate fraction was found to be 697 ± 0.26 mg/100 g dry weight (Gallic acid equivalent, GAE), while the total flavonoid content was 1436 ± 0.47 mg/100 g (QE) **(Fig. S3B)**. Based on the previously discussed qualitative HPLC analysis, the separation of bioactive compounds, and the quantitative determination of flavonoid and phenolic contents, it is evident that the African daisy ethyl acetate fraction is rich in flavonoids, phenolic acids, and quinic acid acyl derivatives. These compounds contribute to the antioxidant and anti-inflammatory activities observed in previously reported species[36-42]. For instance, luteolin, a potent antioxidant, demonstrated significant anti-inflammatory activity in clinical studies by regulating transcription factors such as NF-κB, AP-1, and STAT3[43]. Additionally, quinic acid derivatives like 3,5-O-trans-dicaffeoyl quinic acid methyl ester, 1-O-trans-caffeoyl-5-(7,8-dihydro-7-methoxycaffeoyl) quinic acid, and 5-O-trans-caffeoylquinic acid methyl ester exhibited anti-inflammatory effects by inhibiting nitric oxide production[42]. Phenolic acids, including ferulic, caffeic, syringic, and p-coumaric acids, have shown significant topical anti-inflammatory activity, particularly more so than when administered orally, with their mechanism involving the inhibition of neutrophil migration to the site of inflammation.

Our in vitro and in vivo findings on antioxidant and anti-inflammatory activities, along with metabolite fingerprinting using HPLC and isolation techniques, were compared with previously reported data on Compositae species[4-14]. Species within the Compositae family, including *D. ecklonis*, are rich in antioxidants and anti-inflammatory compounds such as flavonoids, phenolics, sesquiterpene lactones, and phenylpropanoids [6, 13-16]. These compounds and their extracts exhibit anti-inflammatory activity by inhibiting COX and LOX enzymes, as well as reducing cytokine (NO) release from macrophages, along with the inhibition of cyclooxygenase, prostaglandins, and NF-kB release[8-10].

In contrast, the anti-inflammatory response of the African daisy methanol extract was dose-dependent. At the 3rd and 5th hours, the methanol extract at 100 mg/kg resulted in significantly lower inhibition of edema compared to indomethacin, with values of 25.83% and 28.39%, respectively. However, at a dose of 400 mg/kg, the methanol extract's anti-inflammatory response was comparable to that of indomethacin at both time points **(Table 4 & Fig. 4).**

Additionally, the African daisy ethyl acetate fraction at 100 and 400 mg/kg, along with the methanol extract at 400 mg/kg, demonstrated a significant decrease ($P \leq 0.001$) in the dermal area induced by carrageenan five hours post-injection, as shown in **Fig. 4**.

Table 4: Anti-inflammatory effect of *D. ecklonis* ethyl acetate fraction and methanol extract at doses 100 and 400 mg/Kg on carrageenan induced paw edema.

Tested group	Diameter inflammation (mm)			% Edema inhibition		
	1h	3h	5h	1h	3h	5h
Control	4.13±0.12	4.13±0.12	4.13±0.12	---------	----------	---------
Carrageenan	6.75± 0.10	7.82±0.40±0.36	8.24±0.26	--------	----------	---------
Indomethacin	5.14± 0.15*	4.53± 0.10**	4.46±0.20**	23.85	42.07	45.87
D eth 100	5.54 ±0.14*	4.63± 0.25**	4.61±0.20**	17.92	40.79	44.05
D eth 400	5.60 ±0.20*	4.56± 0.30**	4.49±0.60**	17.03	41.68	45.50
D meth 100	6.06± 0.11*	5.80± 0.20**	5.90±0.17**	10.22	25.83	28.39
D meth400	5.3 ±0.1*	4.36 ±0.15**	4.56± 0.1**	21.48	44.24	44.66

D eth 100; ethyl acetate fraction at 100 mg/kg, D eth 400; ethyl acetate fraction at 400 mg/kg, D meth 100; methanol extract at 100 mg/kg, D meth400; methanol extract at 100 mg/kg. $P \leq 0.05$ (*) and $P \leq 0.01$ (**).

As illustrated in **Fig. 5**, the methanol extract of African daisy at both 100 and 400 mg/kg, along with the ethyl acetate fraction at 100 mg/kg, showed no harmful effects on the gastric mucosa. However, the higher dose of the ethyl acetate fraction at 400 mg/kg did exhibit slight mucosal degeneration, possibly due to the increased concentration of acids detected. Our study confirms the safety of the African daisy methanol extract at both doses, as well as the ethyl acetate fraction at 100 mg/kg, on the gastric mucosa and submucosa. In contrast, indomethacin caused focal ulcerative regions, characterized by the shedding of the epithelial lining and the accumulation of necrotic tissue.

Our histopathological findings regarding the rat paw and gastric mucosa confirm the anti-inflammatory and antioxidant properties of *D. ecklonis* extracts previously reported at 100 mg/kg[6]. Additionally, our findings confirm the safety of *D. ecklonis* extracts at both 100 and 400 mg/kg on the gastric mucosa. The toxicity of different *D. ecklonis* extracts has been previously reported, with an LD_{50} of 7-8 g/kg[6]

Conclusion

This study highlights the potent antioxidant and anti-inflammatory properties of the ethyl acetate fraction of *D. ecklonis* (African daisy) and its ability to inhibit histamine release. Thirteen compounds, including flavonoids, phenolics, and quinic acid derivatives, were detected in the fraction. The observed antioxidant and anti-inflammatory activities are likely due to the synergistic effects of key flavonoids, such as kaempferol, luteolin, and apigenin, along with quinic acid derivatives like chlorogenic acid and 4-caffeoyl-5-feruloyl quinic acid methyl ester. These findings support the potential of African daisy as a natural source of therapeutic agents for inflammatory conditions.

List of Abbreviations

HPLC; High Performance Liquid Chromatography, °C; Celsius temperature degree, mg/kg; milligram/kilogram, nm; nanometer, UV/Vis; Ultraviolet/Visible, GAE; Gallic acid Equivalent, QE; Quercetin Equivalent, SD; Standard Deviation, ppm; part per million, g; gram, kg; kilogram, µg; microgram, mL; milliliter, ^{1}HNMR; proton nuclear magnetic resonance,^{13}CNMR; carbon 13 nuclear magnetic resonance, DEPT-135; Distortionless Enhancement by Polarization 135, Fig.; Figure, NF-κB; Nuclear factor kappa-light-chain-enhancer of activated B cells, AP-1; activator protein 1 , STAT3; Signal transducer and activator of transcription 3, NSAIDs; None steroidal anti-inflammatory drugs, IC_{50}; , µM; micromolar, µL; microliter; NO; nitric oxide, NOS; nitric oxide, COX-2; cyclooxygenase 3 enzyme, iNOS; inducible nitric oxide synthase, and PGE2; prostaglandin E synthase 2, CMC; carboxymethyl Cellulose, DPPH; 2,2-diphenyl-1-picrylhydrazyl .

Statements and Declarations

Ethics approval and consent to participate

Author's animal experiment was approved by the Institutional Animal Care and Use Committee of the Faculty of Medicine, Sohag University (Approved No. 12-7-7/2024-01)

(**Fig. S9)**. All methods are reported in accordance with ARRIVE guidelines (https://arriveguidelines.org). This experiment was carried out in accordance with relevant guidelines and regulations.

Plant ethics - The plant was kindly identified by Dr. Mahmoud Abdelhady Hassan, a professor of aromatic plants and horticulture. Voucher specimens were deposited in the herbarium of the Pharmacognosy Department at the Faculty of Pharmacy, Assiut University, Egypt, with the following voucher numbers: 2221984 (*D. ecklonis*), 2221985 (*A. aurantiaca*), and 2221983 (*S. cruentus*). This study was carried out in accordance with relevant guidelines and regulations. Permission to collect plant material was obtained from Plant Garden of Horticulture and Aromatics at the Faculty of Agriculture, Minia University, Egypt.

Acknowledgement

The authors express their sincere thanks to Dr. Mahmoud Abdelhady Hassan, a professor of aromatic plants and horticulture, for his kind participation in the taxonomic identification of the plant species in the current study.

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Bull. Pharm. Sci., Assiut University, Vol. 48, Issue 2, 2025, pp. 969-986.

نشرة العلوم الصيدليـــة
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BULL. PHARM. SCI. Assiut Univ.

النشاط المضاد للالتهابات القوي لزهرة الأقحوان الأفريقية الغنية بالبوليفينولات ومشتقات حمض الكينك على وذمة كف الفأر المستحثة بالكارجينان

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تهدف الدراسة الحالية علي دراسة الأنشطة المضادة للالتهابات والأكسدة لخلاصات الأسيتات الإيثيلية والكحولية من زهرة الأقحوان الأفريقية، بالإضافة إلى دراسة التركيب الكيميائي وتأثيرها الامن علي الأنسجة المعدية مقارنة بالإندوميثاسين (مضادات الالتهاب غير الستيرويدية). تم إجراء الفحوصات النسيجية على كف الأقدام والأنسجة المعدية للفئران المعالجة بخلاصات الاسيتات الايثيلية والميثانول لاقحوان أفريقيا، بالإضافة إلى مقارنتها بالإندوميثاسين كمركب مرجعي ايجابي. تم إجراء قياسات كمية لسمك الطبقات الجلدية في جميع المجموعات باستخدام برنامج ImageJ. تم استخدام تقنيات HPLC والعزل للتوصيف الكيميائي النباتي لجزء الأسيتات الإيثيلية من زهرة الأقحوان الأفريقية. تم استخدام اختباري DPPH و MTT لتقييم خصائص مضادات الأكسدة وإطلاق مضادات الهيستامين من مستخلصات زهرة الأقحوان.

أظهر مستخلص الأسيتات الإيثيلية من زهرة الديمورفوتيكا نشاطًا مضادًا للالتهابات كبيرًا مشابهًا لذلك الذي يظهره الإندوميثاسين. لم تُلاحظ أي تغيرات مرضية كبيرة في الأنسجة المعدية للجرذان المعالجة بمستخلصات الأسيتات الإيثيلية والكحولية. تم التعرف علي ثلاثة عشر بوليفينول من فئات مختلفة في المستخلص الإيثيلي، بما في ذلك كيمبفيرول، لوتولين، أبيجينين، حمض الكلوروجينيك، و٤-كافيل-٥-فيرولويك ميثيل إستير حمض الكينك. ساهم المحتوى العالي من الفينولات والفلافونويدات في نشاطه القوي المضاد للأكسدة، والمضاد للهيستامين، والمضاد للالتهابات. خلصت دراستنا إلى أن مستخلص الأسيتات الإيثيلية لنبات الاقحوان الافريقي له نشاطات مضادة للالتهابات والأكسدة ومضادة للهيستامين بشكل كبير علي فئران التجارب المعملية وكذلك الاختبارات المخبرية. تُعزى هذه التأثيرات القوية إلى التفاعلات التآزرية للفينولات والفلافونويدات، مثل كيمبفيرول، لوتولين، أبيجينين، حمض الكلوروجينيك، و٤-كافيل-٥-فيرولويك ميثيل إستير حمض الكينك.