Italian Journal of Food Science, 2024; 36 (2): 38–47

Evaluation of analgesic, antioxidant, and anti-inflammatory potential of *Dianthus crinitus* using mice as a research animal

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Received: 12 December 2023; Accepted: 30 January 2024; Published: 1 April 2024

ORIGINAL ARTICLE

Abstract

In the past, natural resources were used as best therapeutic sources for different diseases. Among several herbal resources, certain plant families are known for their analgesic activities, such as *Dianthus crinitus*, which showed a good curative potential. The mentioned plant has been used traditionally for the healing of several illnesses such as pain, inflammation, injuries, etc. The current study, therefore, evaluated the analgesic and anti-antioxidant activities of ethanolic extracts of leaves, flowers, and seeds of *Dianthus crinitus* in mice. For analgesic activity, acetic acid and a hot plate thermal stimulation method were used to induce writhes in mice, followed by a tail emersion process. Results showed that the extract demonstrated analgesic potential in mice, signifying both peripheral and central modes of action. Furthermore, extract also significantly inhibited the rat paw edema triggered by carrageenan seaweed and dextran as revealed by neutrophil migration toward the peritoneal cavities of animals and enhanced vascular permeability. The data suggested that the extract exhibited a dose-dependent antioxidant action. These preliminary observations provided support for further exploration and justification for the potential development of a new class of analgesic and anti-inflammatory medicines from the constituents of *Dianthus crinitus* extract.

Keywords: analgesic; anti-inflammatory; antioxidant; aqueous extract; *Dianthus crinitus*; potential

Introduction

For centuries, plants have offered mankind a natural basis for medications (Van Wyk and Wink, 2018). Plant-based drugs are used as indigenous cultural and traditional cures (Jahangirian *et al.*, 2019). According to the World Health Organization (WHO), 70% of the world's population uses about 35–70 plant species for therapeutic purposes (Ammara *et al.*, 2023; Areej *et al.*, 2023; Gul *et al.*, 2023; Iram *et al.*, 2023; Monisa *et al.*, 2023; Syed *et al.*, 2023; Waqas *et al.*, 2024), because these plant species contain thousands of phytochemicals with medicinal properties and without adverse reactions (Arnold 2013). Moreover, 25% of the ingredients used in pharmaceuticals are derived from medicinal plants (Robbers *et al.*, 1996). Several studies have demonstrated that herbal medicines

ISSN 1120-1770 online, DOI 10.15586/ijfs.v36i2.2492

from the Vedic era have been used for treating various disorders, such as heart diseases, cancer, digestive system diseases, central nervous system (CNS) diseases, diabetes, and inflammatory diseases (Ahmad *et al.*, 2023a; Aqib *et al.*, 2023; Ayesha *et al.*, 2022; Aziz *et al.*, 2023; Ejaz *et al.*, 2023; Hussain *et al.*, 2023; Naveed *et al.*, 2022; Nureen *et al.*, 2023; Rauf *et al.*, 2023; Zawar *et al.*, 2023).

Pain is one of the most common conditions reported globally (Nicholas *et al.*, 2019). Pain has been classified by anatomic location, body system, duration, severity, frequency, and etiology (Kasman and Duruöz, 2019). Under these stressed conditions, human body yields more reactive oxygen species (ROS) (e.g., hydroxyl radicals, superoxide anion radicals, and hydrogen peroxide) (Mohammadi, 2019) than enzymatic antioxidants (e.g., glutathione peroxidase [GPx], superoxide dismutase [SOD], and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid [vitamin C], glutathione, alpha-tocopherol [vitamin E], flavonoids, and carotenoids) (Strycharz-Dudziak *et al.*, 2020).

The imbalance between antioxidants and ROS results in cell damage and cell death. Antioxidants are the substances that reduce and eliminate these reactive free radicals (Adebayo *et al.*, 2020). Deficiency or absence of antioxidants in the body leads to the development of degenerative diseases, such as cancers, cardiovascular disease (Teleanu *et al.*, 2019), neurodegenerative diseases, Alzheimer's disease (Riaz *et al.*, 2023; Saleem *et al.*, 2020), and inflammatory diseases (Li *et al.*, 2020).

Various medicinal herbs are used in a traditional way to heal various ailments, and this practice is as old as 500 years (Buso *et al.*, 2020). The widespread usage and assessment of natural products for decades helped in the discovery of important healing agents, and currently, these agents are used in modern medications (Gunjan *et al.*, 2015).

Recently, *Dianthus crinitus* has been reported for its ethno-pharmacological, anti-catarrhal, antipyretic, antispasmodic, bronchi dilatator, diuretic, expectorant, and diarrheal cure properties (Ahmad *et al.*, 2023b; Ghamari *et al.*, 2017). Thus, in the current study, *Dianthus crinitus* was selected for its analgesic and antioxidant activities in a selected animal model.

Materials and Methods

Collection of plant material

The leaves, seeds, and flowers of *Dianthus crinitus* were collected from District Swat in June 2020. The plant was recognized and authenticated by Prof. Muhammad Nisar. The plant materials comprised water and shade-dried plant parts, which were crunched to powdered form using automatic mortar.

Extraction

Leaves (480 g), seeds (543 g), and flowers (234 g) of the plant were mixed separately with ethanol (1500 mL) for 7 days. The ethanolic mixture was filtered. Using rotatory evaporator, the filtrate was vaporized under low pressure and at 40°C temperature. After complete vaporization of the solvent, crude ethanolic extracts of leaves, greenish in hue (1600 mg), seeds, yellowish in color (211 mg), and flowers, light pink in color (105 mg), were collected. For further usage, these crude ethanolic extracts were stored in glass containers at 4°C.

Acute test

Acute toxicity study was carried out using albino mice as test animals. Eight mice were categorized into control and test groups. Animals were administered with different doses of plant extracts in the range of 200–1500 mg/kg body weight. Then the animals were observed for next 52 h for any abnormal manifestations of mortality and lethality (Hosseinzadeh *et al.*, 2000).

Analgesic study

The analgesic potential of crude ethanolic extracts of leaves, flowers, and seeds of the plant at various doses were studied using mice as an experimental model by performing the following three procedures (Hassan *et al.*, 2008, Owoyele *et al.*, 2008).

Licking paw edema

Formalin (2.6%) was mixed with 15-µL (v/v) purified water and was used to induce licking of the paw to study the nociceptive behavior of animals. Different doses of extracts were administered orally after 25 min of formalin ingestion. The nociceptive behavior of animals, including licking and biting of paws, was noted after administration of formalin. The neurogenic phase was of 5 min and the late phase or inflammatory phase of nociceptive activities was of 15–30 min.

Writhing test

To induce writhing in mice, acetic acid was used to assess the anti-nociceptive activity of the plant. Acetic acid (0.7% and 10 mL/kg body weight) was injected intraperitoneally (IP) in each animal. After 30 min of administration of acetic acid, different doses of ethanolic extracts were given orally as follows:

Group I mice received 0.6% Tween 80 solution (10 mL/kg), which were treated as control animals.

Group II mice were exposed to diclofenac sodium (standard drug) at a dose of 100 mg/kg body weight.
Groups III animals were given ethanolic extracts of leaves, flowers, and seeds at a dose of 75 mg/kg body weight.
Group IV animals were given ethanolic extracts of leaves, flowers, and seeds at a dose of 100 mg/kg body weight.
Group V animals were given ethanolic extracts of leaves, flowers, and seeds at a dose of 200 mg/kg body weight.

The writhing was observed for 5–30 min of ingestion of acetic acid.

Tail immersion assay

This assay was carried out to evaluate the anti-nociceptive effect of ethanolic extracts of plant leaves, flowers, and seeds. Briefly, the tail (2 cm) of each animal was dipped into hot water (54±0.5°C), followed by ingestion of 75 mg/kg and 150 mg/kg body weight of extracts. Mice response (the time spent during withdrawal of tail from hot water) or reaction time) was recorded at interval of 30, 60, 90 and 120 min after extract administration.

Antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH; 15 mg) stock solution was prepared. Mother solution of the plant extract was prepared in methanol. Stock solution, 50, 100, 200, 300, 700 µg /mL, was separated in tubes and expressed in triplicate. The same process was repeated for ascorbic acid (as a standard) using 1700 Shimadzu Japan) at 517 nm for absorbance (Ahmad, Ullah *et al.*, 2015). The proportion of free radical scavenging activity was calculated using the following equation:

$$(X) = (A) - (B) \div (A) \times 100,$$

where (X) is the free radical scavenging activity, (A) is the control absorbance, and (B) is the sample absorbance.

Results

Phytochemical Screening

The crude ethanolic extracts of leaves, seeds, and flowers of *Dianthus crinitus* were screened for their phytochemical constituents, including phenols, tannins, saponins, flavonoids, steroids, and terpenoids (Table 1). The results showed that the plant is highly rich in phytochemical constituents. Crude extract of leaves contained a large quantity of phenols and terpenoids. Likewise, the crude extract of seeds was found having high levels of tannins and steroids but lacked flavonoids. The flower extract had all the mentioned phytochemicals, with high levels of flavonoids but no saponins. Analysis of phytoconstituents of the plant demonstrated that it was rich in secondary metabolites.

Antioxidant Activity

The antioxidant activity of crude ethanolic extracts of leaves, seeds, and flowers of *Dianthus crinitus* was analyzed using DPPH free-radical inhibition test. It was found that the extracts in a dose-dependent manner markedly showed free radical inhibition. Ethanolic extract of leaves inhibited by 41.30±1.43 (42.66 %), 50.41±1.32 (49.96 %), 64.18±0.57 (61.71 %) , 66.51±1.33 (64.70 %) and 70.21±1.63 (70.00 %) (Figure 1 and Table 2).

Inhibition was measured at concentrations of 50, 100, 200, 300, and 700 µg/mL. The IC_{50} value of ethanolic extract of leaves was found as 112 µg/mL. Likewise, the crude extract of flowers showed the following scavenging activity of free radicals at above-mentioned dosages: 34.17±1.22 (32.66%), 43.32±0.43 (41.96%), 45.32±0.71(42%), 55.64±1.12(54.70%), and 67.55±0.53 (65.00%). The crude ethanolic extract of seeds of *Dianthus crinitus* showed nearly the same antioxidant activity, that is, 41.00±0.00 (40.36%), 56.22±0.28 (51.76%), 56.29±1.541 (52%), 62.46±0.52 (63.60%), and 67.00±2.26(67.12%). DPPH inhibition at the same concentration described for leaves and flower, with an IC_{50} of

Table 1. Phytochemical screening of ethanolic extracts of leaves, seeds, and flowers of *Dianthus crinitus* plant.

Extracts	Phytochemicals					
	Tannins	Saponins	Flavonoids	Phenolics	Steroids	Terpenoids
Leaves	**	**	**	***	**	***
Flower	*	**	****	****	*	***
Seed	****	N	N	**	***	**

*Present, N = absent.

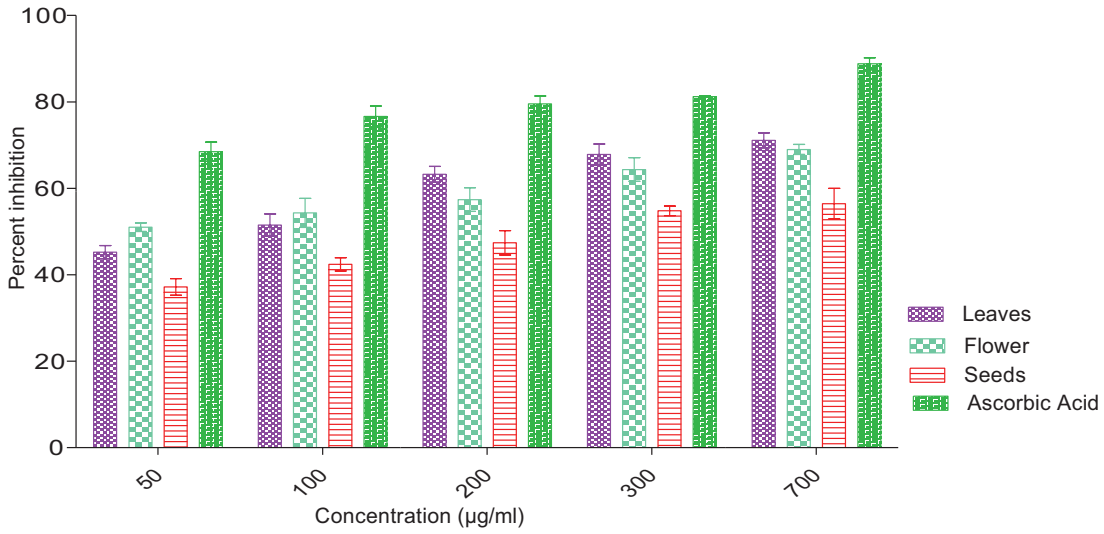

Figure 1. Percentage inhibition activity of ethanolic extracts of *Dianthus crinitus* leaves, seeds, and flowers based on DPPH solution, compared to ascorbic acid as a standard.

Table 2. The IC_{50} values of crude ethanolic extracts of leaves, flower, and seeds of *Dianthus crinitus* against DPPH free radical inhibition.

Extracts	IC_{50} (µg/mL)	Inhibition (%)
Leaf	44	41
Flower	78	74
Seeds	69	62
Ascorbic acid	<0.2	84

(IC_{50}): Determination of inhibitory concentration.

Table 3. Antioxidant activity of ethanolic crude extracts of leaves, flowers, and seeds of *Dianthus crinitus* versus DPPH free-radical scavenging assay.

Leaves extract		
Concentrations (µg/mL)	**Inhibition (%)**	**Mean±SEM**
50	42.66	41.30±1.43
100	49.96	50.41±1.32
200	61.71	64.18±0.57
300	64.70	66.51±1.33
700	70.00	70.21±1.63
Flowers extract		
Concentrations (µg/mL)	**Inhibition (%)**	**Mean±SEM**
50	32.46	34.17±1.22
100	41.33	43.32±0.43
200	42	45.32±0.71
300	54.40	55.64±1.12
700	65.23	67.55±0.53
Seeds extract		
Concentration (µg/mL)	**Inhibition (%)**	**Mean±SEM**
50	40.36	41.00±0.00
100	51.76	56.22±0.28
200	52	56.29±1.541
300	63.60	62.46±0.52
700	67.12	67.00±2.26

51 µg/mL. Ascorbic acid used as a standard showed >80% free radical inhibition at a concentration of 100 µg/mL, with IC_{50} value < 0.1 µg/mL (Figure 1 and Tables 2 and 3).

Acute toxicity

The acute toxicity test is performed to determine lethality and safe doses of a medicinal plant using animals before *in vivo* pharmacological studies. Thus, acute toxicity tests in animals confirm the safe dosage for further animal investigations. Crude ethanolic extracts of leaves, seeds, and flowers of *Dianthus crinitus* were investigated for its acute toxicity using mice as test animals in two phases (Table 4). In the first phase, mice were exposed to ethanolic extracts of leaves, flowers, and seeds intraperitoneally at concentrations of 50, 100, and 1000 mg/kg body weight and examined for toxicity or any harmful reaction. In the first phase, 1000 mg/kg of plant different extracts produced no toxicity and hence considered as a safe concentration. In the second phase, mice received 1050, 1400, and 1550 mg/kg of ethanolic extract samples. The crude ethanolic extracts of flowers and seeds were determined as safe at 1000-mg/kg body weight intraperitoneally.

Ethanolic extract of leaves showed altered results than flower and seed samples. At 1050-mg/kg body weight IP dose, 50% of mice got affected with maximum mortality. At 1100-mg/kg IP dose, all animals perished. The results

showed that crude extract seed samples were nontoxic, harmless, and safe up to 1200 mg/kg body weight IP dose, while ethanolic extract of leave samples were safe up to 1000-mg/kg body weight IP dose.

Analgesic Activity

To investigate the analgesic activity of *Dianthus crinitus* leaves, flowers and seeds extracts, different test models, such as formalin-induced paw licking test, tail immersion test, and abdominal writhing test, were analyzed. Acetic acid was used to induce abdominal writhes in mice, and this was followed by administering leaves, flower and seed extracts at doses of 100-mg/kg and 300-mg/kg body weight. Leaves extract inhibit the acetic acid-induced abdominal writhes in mice (first phase 48%–57%) and (Analgesic activity 55% and 73%) at the mentioned doses, respectively. Seeds extract produced 66% and 72%, while flowers extract produced 59% and 75%, inhibition in abdominal writhing caused by acetic acid in mice at 100-mg/kg and 300-mg/kg body weight, respectively. Diclofenac sodium, used as a standard analgesic at a dose of 10-mg/kg body weight, displayed 81% activity (Table 5).

Concerning paw licking caused by formalin, the analgesic effect of crude ethanolic extract of leaves produced 40% anti-nociceptive effect in phase 1st and 2nd of assay. Likewise, flower extract at a dose of 300 mg/kg caused 58.40% analgesic effect during the 1st and 2nd phase of assay. Furthermore, seeds extract was observed as having 47% and 54% inhibition response during the 1st and 2nd phase of assay. Flower extract revealed 43% and 56% protection of formalin-cased paw-licking response in the 1st and 2nd phase, while 60 and 64% inhibition were observed at 300 mg/kg in phase 1st and 2nd. Meanwhile, indomethacin, used as a standard analgesic, showed 60% and 74% activity for formalin-caused paw-licking response during the 1st and 2nd phase (Table 6).

Results of tail immersion test, shown in Table 7, indicated that the ethanolic extract of leaves (75 mg/kg and 150 mg/kg) produced significant reduction in analgesic response. The maximum analgesic effect of leaves extract was observed at a dose of 150 mg/kg. Latency increased by 52.11% ($p < 0.01$, n=5). Similarly, flower extract at (150 mg/kg) exhibited major effect, that is, 55.25% inhibition at 75 min ($p < 0.001$). Increase in the latency rate (58%) of tail flickering was observed by administering a high dose of seeds extract. Powerful activity (84%) was observed for tramadol (a centrally acting opioid) at 60 min after the treatment ($p < 0.001$). Mice exposed to naloxone

Table 4. Acute toxicity test observations of ethanolic extracts of flowers, seeds, and leaves of *Dianthus crinitus* in mice.

Treatment (mg/kg of body weight)			
Phase 1st	Group 1 (50 mg)	Group 2 (100 mg)	Group 3 (1000 mg)
Seeds	Vigorous	Healthy	Healthy
Flower	Active and strong	Strong and active	Alive
Leaves	Active and vigorous	Active and healthy	Alive
Phase 2nd	Group 1 (1050 mg)	Group 2 (1100 mg)	Group 3 (1200 mg)
Seeds	Alive+lethargic	Alive+lethargic	Alive+lethargic
Flower	Alive+lethargic	Alive+lethargic	Alive+lethargic
Leaves	50% died	All died	All died

Table 5. Presenting assessment of analgesic activity of *Dianthus crinitus* using acetic acid-induced analgesic activity.

Treatment/dose	No of writhes (sec)		Inhibition (%)	
	Phase 1st Mean±SEM (n=5)	Phase 2nd Mean±SEM (n=5)	Phase 1st Mean±SEM (n=5)	Phase 2nd Mean±SEM (n=5)
Control	60.02±1.54	73.00±1.430	--	--
Leaves 100 mg	41.22±1.12**	34.23±1.519**	18.60	48.65
300 mg	31.66±1.125**	26.23±1.668***	35.71	56.40
Flower 100 mg	50.01±1.65**	58.45±1.435**	0.049	19.43
300 mg	50±1.411***	53.73±1.029***	0.069	24.93
Seeds 100 mg	28.96±1.55**	36.65±1.541***	43.11	53.90
300 mg	20.3±1.05**	26.75±0.95**	58.42	62.19
Indomethacin (10 mg)	19.83±1.55**	16. 46±1.541***	61.36	76.25

Note. **$p < 0.01$ and ***$p < 0.001$, compared to the control group (one-way ANOVA followed by Dunnetts [Data analysis Test)] compare all vs. the control test).

Table 6. Assessment of analgesic activity of *Dianthus crinitus* in mice using paw-licking response induced by formalin.

Treatment/dose (mg/kg body weight)	Paw-licking time (sec)		Inhibition (%)	
	1st Phase	2nd Phase	1st Phase	2nd Phase
Control 2% Tween 80	34.32±1.17	60.21±1.32	----	--
Leaves 100	22.24±1.63***	21.98±1.81***	56.51	64.91
300	18.24±1.13**	19.22±1.41**	55.82	71.34
Flower 100	34.32±1.17	60.21±1.32	----	--
300	22.24±1.63***	21.98±1.81***	56.51	64.91
seed 100	14.32±1.17	23.21±1.32	42.45	51.43
300	22.24±1.63***	21.98±1.81***	56.51	64.91
Indomethacin 10 mg	34.11±1.30	17.21±1.31***	16.95	74.71

Note. $^{**}p < 0.01$ and $^{***}p < 0.001$, compared to the control group (one-way ANOVA followed by Dunnetts: compare all vs. the control test).

Table 7. Assessment of analgesic activity of *Dianthus crinitus* leaves, flowers, and seeds extract in mice using formalin-induced tail flickering response.

	Tail flickering (sec)/response (%)				
Treatment (mg/kg)	15 min	30 min	60 min	90 min	120 mi
Control	0.97±1.31	1.08±1.42	1.04±1.52	1.03±1.84	1.06±1.51
Tramadol 30	1.64±1.24** 42.23%	2.31±1.63** 51.37%	4.90±1.39*** 76.96%	6.72±3.09*** 84.70%	5.86±1.50*** 78.92%
Leaves ethanolic extract 75	1.53±1.11 12.27%	1.54±1.35* 18.40%	1.73±1.44** 37.19%	1.73±1.31** 42.02%	2.4±1.33** 52.11%
150	1.27±1.29* 23.80%	1.45±2.48* 26.51%	1.71±1.26** 42.16%	2.35±1.22** 54.60%	2.68±1.61*** 56.25%
Flower ethanolic extract 75	1.07±1.42 10.23%	1.18±1.36 12.34%	1.21±1.41 19.34%	1.28±1.57 34.23%	1.19±1.38 38.23%
150	1.05±1.86 9.23%	1.11±1.53 13.45%	1.19±1.41 14.34%	1.21±1.32 19.34%	1.21±1.51 44.45%
seed ethanolic extract 75	1.20±1.12 10.12%	1.71±1.35* 14.40%	1.51±1.64** 15.22%	1.97±1.40** 43.02%	2.46±1.42** 56.11%
150	1.23±1.25* 22.70%	1.50±2.38* 28.41%	1.89±1.56** 46.16%	2.58±1.32** 56.40%	2.89±1.41*** 58.38%

Note. $^{**}p < 0.01$ and $^{***}p < 0.001$, compared to the control group (one-way ANOVA followed by Dunnetts: compare all vs. the control test).

demonstrated significant decrease in analgesic action. Crude ethanolic extract of leaves + flower caused better analgesic effect and showed anti-nociceptive potential. This suggested that the plant contained high levels of flavonoids and tannins. Hence, the primary phytochemical results showed that extracts had high levels of tannins and flavonoids, which could be responsible for anti-inflammatory and anti-nociceptive effects.

Discussion

Analysis of phytoconstituents is one of the major methods used for the identification of secondary metabolites of a medicinal herb. Natural metabolites comprise steroids and a glycone (sugar group) bonded by S-glycosidic bond with varied biological activities (Abdelrahman and Jogaiah, 2020). Medicinal plants are known for their therapeutic value because of the phytoconstituents responsible for various medicinal activities (Roger *et al.*, 2015). Regarding pharmacological efficacy, the most significant phytoconstituent is saponins (Batiha Saber, 2002). Saponins, used for curing various infections, are known for antimicrobial, antioxidant, antidiabetic, cytotoxic, antispasmodic, antitumor and anthelmintic properties (Barua *et al.*, 2018). These are also extensively used in various investigations such as anthelmintic, anticancer, and insecticidal conditions (Joshi *et al.*, 2020).

In the current study, normal to moderate antioxidant activities of ethanolic extracts of leaves, seeds, and flowers of *Dianthus crinitus* was examined. These activities could be due to the presence of phytochemicals, especially phenols. Similar results were presented by Shah *et al.* (2015), who reported that phenols showed efficiency in many biological actions, of which antioxidant activity was significant. Marked antioxidant activity of phenolics was carried out via redox reaction, with metal chelation affinity (Rice-Evans *et al.*, 1995). Several studies have been conducted for investigating correlation between phenolic molecules and their antioxidant potential (Wang *et al.*, 1999; Zainol *et al.*, 2003).

This study showed that the ethanolic extract of *Dianthus crinitus* leaves diminished writhing action. According to Rahman *et al.* (2011), in (AA) acetic acid triggered writhing reaction in which the released of pain mediators or it directly excite the acid sensitive receptors. Ethanolic extracts of *Dianthus crinitus* leaves, flowers, and seeds exhibited dose-dependent reduction in the writhing responses of mice; this showed that it might be exerting peripheral activity on the nervous system (Su *et al.*, 2023; Huang *et al.*, 2023; Hu *et al.*, 2022; Qiu *et al.*, 2018; Song *et al.*, 2018; Bao *et al.*, 2016).

It was suggested that the extracts had analgesic potential, which was nearly equivalent to that of diclofenac sodium. The analgesic action of the extracts could be either due to the activation of visceral receptors sensitive to acetic acid or the prevention of conduction of painful messages to the central nervous system (Hosseinzadeh and Younesi, 2002). The pain caused by acetic acid was due to the release of endogenous ingredients, such as prostaglandins, that stimulate pain nerve terminals (Raj, 1996). When prostaglandin E2 (PGE2) is released, the nerve terminals react with the molecules, picking up and directing the pain message through nervous system to the brain (Franzetti *et al.*, 2009; Schneider *et al.*, 2011).

Findings of the current investigation are in line with the results of Yaakob *et al.* (2013), who examined the analgesic and anti-inflammatory potential of ethanolic leaves extract of medicinal plants. Drug that protect from the first phase of formalin assay, may be either the effect of medicine, while those drugs that inhibit the second phase may relieve the pain (Tjølsen *et al.*, 1992; Yusuf *et al.*, 2015). Usually the acetic acid-triggered writhing assay is performed as a model during visceral pain, and is used in the development of new analgesic drug (Elisabetsky *et al.*, 1995; Vyklicky, 1979). Similarly, tail flick test is a thermal induced pain exhibit sedative action, which is sensitive to opioid receptors (Abbott and Young, 1988). The ability of *Dianthus crinitus* extracts to inhibit reaction latency to thermally triggered pain in mice confirmed that it was a fundamental analgesic in action, which could be due to the presence of bioactive compounds (Liu et al., 2024; Niu *et al.*, 2023; Bittar *et al.*, 2000; Das *et al.*, 1989; Pateh *et al.*, 2011; Starec *et al.*, 1988; Yusuf, 2014).

Conclusion

The presence of medicinally important secondary metabolites was confirmed through the screening of phytochemical constituents of *Dianthus crinitus*. Further, the plant extract displayed best analgesic, anti-inflammatory, and antioxidant properties because of the presence of vital metabolites, which confirmed the plant's rich therapeutic impact. Further exploration and isolation of pharmacologically active compounds is required for developing novel drugs.

Conflict of interest

The authors declared no conflict of interest.

Ethical approval

The ethical approval for this study was granted by Department of Pharmacy, University of Malakand under reference No. Pharm/23/4168.

Acknowledgment

The authors extend their appreciation to the Deputyship for Research and Innovation, "Ministry of Education" in Saudi Arabia for funding this research (IFKSUOR3- 291-1).

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