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Phytochemical Screening and Evaluation of Analgesic Activity of Capparis cartilaginea leafExtract in Experimental Animal Model

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Abstract

Background: Pain remains a major therapeutic challenge, and the use of conventional analgesics is often limited by undesirable adverse effects. *Capparis cartilaginea* is traditionally employed in the Arabian Peninsula to alleviate pain and inflammatory disorders.

Aim: This study aimed to investigate the Phytochemical Profile and Analgesic Activity of *Capparis cartilaginea* leaf Extract.

Methods: Preliminary phytochemical screening was conducted to identify the main classes of bioactive compounds. The analgesic activity of the ethanolic leaf extract (200 and 400 mg/kg) was evaluated in male albino rats using the acetic acid–induced writhing test as a model of peripheral nociception.

Results: Phytochemical analysis revealed the presence of carbohydrates, fixed oils, glycosides, anthraquinones, phenolic compounds (including tannins and flavonoids), saponins, and gum/mucilage. . The extract produced a significant ($P < 0.05$), dose-dependent reduction in the number of writhes in the acetic acid model, supporting a peripheral analgesic effect.

Conclusion: The ethanolic extract of *C. cartilaginea* leaves, enriched with flavonoids, tannins, glycosides, saponins, and anthraquinones, showed a clear dose-dependent antinociceptive activity in the acetic acid writhing assay, with the 400 mg/kg dose exhibiting an effect comparable to diclofenac. These findings suggest that inhibition of inflammatory mediators such as prostaglandins and bradykinin may underlie its analgesic action, thereby providing pharmacological support for the traditional use of *C. cartilaginea* in pain management.

Article Info

Keywords:
Capparis cartilaginea , Writhing test , Analgesic activity , Diclofenac, Phytochemical Screening.

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1. INTRODUCTION

Pain is a complex sensory and emotional experience that plays a key protective role and represents a major clinical problem worldwide. Despite its protective role, pain remains one of the most common clinical complaints and a major cause of reduced quality of life and functional impairment [1, 2]. Although opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for pain relief, their therapeutic use is limited by adverse effects, such as gastrointestinal, renal, and cardiovascular complications, as well as the risk of dependence [3, 4].

Medicinal plants have long been recognized as important reservoirs of bioactive secondary metabolites with potential pharmacological properties, including anti-inflammatory, antioxidant, and analgesic activities. Numerous phytoconstituents, such as flavonoids, tannins, saponins, glycosides, and phenolic compounds, have been reported to modulate nociceptive pathways and inflammatory mediators involved in pain perception [5–8]. *Capparis cartilaginea* Decne. (family: Capparaceae) is a medicinal plant distributed in Yemen and other regions of the Arabian Peninsula. In traditional medicine, different parts of this plant have been used to manage inflammatory and pain-related conditions. Previous phytochemical and pharmacological studies on *C. cartilaginea* and re-

lated *Capparis* species have demonstrated the presence of diverse secondary metabolites and several biological activities, including antioxidant, anti-inflammatory, and antidiabetic effects [9–12].

2. MATERIALS AND METHODS

2.1. Chemicals , Reagents and Drugs

Ethanol (96%) was obtained from Umcanol (Yemen). Potassium hydroxide, ethanolic vanillin, and glacial acetic acid were purchased from Uni-Chem Co. (India) and WinLab (UK), respectively. Hydrochloric acid was obtained from Scharlau (Spain), sulfuric acid from Roth (Germany), potassium iodide from Shantou (China), iodine from BDH (England), Mayer's reagent from Gazi Stain (India), and Dragendorff's reagent from Pure Chemical (Yemen). Sodium diclofenac was obtained from Denk Pharma (Germany).

2.2. Plant Materials

Fresh leaves of *C. cartilaginea* were collected from the Hajjah Governorate, Yemen, in March 2024. The plant was taxonomically identified by Dr. Ibraheem Hassan at the Faculty of Science, Sana'a University, Yemen.

2.3. Preparation of the Extract

Fresh leaves (900 g) of *C. cartilaginea* were thoroughly washed, shade-dried at room temperature, and pulverized into a coarse powder. The dried powdered material was extracted with 96% ethanol via maceration, followed by filtration. The filtrate was concentrated to dryness under reduced pressure using a rotary evaporator. The dried extract was weighed and stored in airtight containers at room temperature until further use. The final percentage yield of the leaves was calculated using the following equation:

Yield (%) = (Weight of dried extract/Weight of dried plant powder) × 100

2.4. Experimental Animals

Adult male albino rats weighing 150–250 g were obtained from the Animal House, Faculty of Science, Sana'a University, Yemen. The animals were housed in polypropylene cages under standard laboratory conditions (23 ± 2℃, 12 h light/12 h dark cycle) with free access to standard diet and water. The animals were acclimatized for at least 48 h before the experiment began. All experimental procedures were conducted in accordance with the institutional guidelines for the care and use of laboratory animals and were approved by the Institutional Ethical Committee, Faculty of Medicine and Health Sciences, Sana'a University, Yemen (Approval Code: 26/10-7).

2.5. Phytochemical Screening

The ethanolic leaf extract of *C. cartilaginea* was subjected to preliminary qualitative phytochemical screening to identify the presence of major classes of secondary metabolites, including alkaloids, carbohydrates, fixed oils, glycosides, anthraquinones, phenolic compounds, flavonoids, saponins, phytosterols, proteins, and gum/mucilage, using standard phytochemical methods as follows. [6]

Test of Alkaloids: To 2 mL of the methanolic filtrate, 1.5 mL of 1% hydrochloric acid was added, and the mixture was heated in a water bath. Thereafter, a few drops of Mayer's, Wagner's, or Dragendorff's reagents were added separately. The formation of an orange or reddish-brown precipitate indicated the presence of alkaloids.

Saponin test**:** A foaming test was performed using 2 g of powdered plant material in either aqueous or alcoholic extract. The persistence of frothing after shaking indicates the presence of saponins. A few drops of olive oil were added to confirm the results.

Glycoside test**:** Two milliliters of alcoholic filtrate were mixed with 1 mL of glacial acetic acid containing one or two drops of ferric chloride, followed by the careful addition of 1 mL of concentrated sulfuric acid. The appearance of a brown ring at the interface indicates the presence of cardiac glycosides.

Terpene analysis**:** Two milliliters of alcoholic extract were mixed with 5 mL of chloroform and 2 mL of acetic anhydride. Concentrated sulfuric acid was then carefully added to form a new layer. The appearance of a reddish-brown color at the interface indicated the presence of terpenes.

Test for Flavonoids: Two grams of plant material were extracted with 10 mL of either water or alcohol. To the filtrate (2 mL), a few drops of concentrated hydrochloric acid and 0.5 g of zinc or magnesium turnings were added. The development of a magenta red or pink color after approximately 3 min indicated the presence of flavonoids in the extract.

Test for Phenolic Compounds

To 2 mL of either the aqueous or alcoholic extract, 1 mL of 1% ferric chloride solution was added. The appearance of blue or green coloration indicates the presence of phenolic compounds.

Test for Carbohydrates

Benedict's and Fehling's tests were performed to confirm the presence of reducing sugars and carbohydrates in the extract.

2.6. Evaluation of Antinociceptive Activity

The antinociceptive activity of the ethanolic leaf extract was evaluated using the acetic acid–induced abdominal writhing test in rats, which is a well-established model

of peripheral nociception [13, 14] The animals were randomly divided into four experimental groups (n = 6 per group) as follows: **Group I:** Distilled water (negative control), **Group II:** Diclofenac sodium (20 mg/kg, i.p.) (positive control), **Group III:** *C. cartilaginea* extract (200 mg/kg, i.p.), **Group IV:** *C. cartilaginea* extract (400 mg/kg, i.p.). All treatments were administered intraperitoneally (i.p.) 30 min before the intraperitoneal injection of 0.6% acetic acid solution (10 mL/kg), which was used to induce nociceptive writhing. Following acetic acid administration, each animal was individually observed, and the total number of writhing responses was counted over 30 min. Writhing was characterized by abdominal constriction accompanied by hind limb stretching. A reduction in the number of writhing episodes was considered indicative of peripheral antinociceptive activity in mice. The selected doses of the ethanolic extract (200 and 400 mg/kg) were chosen based on preliminary experimental observations and previous pharmacological studies involving medicinal plant extracts with potential analgesic activity.

2.7. Statistical Analysis

Data are expressed as mean ± SD. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test using GraphPad Prism software (version 8.0; GraphPad Software, USA). Statistical significance was set at $P < 0.05$.

3. RESULTS

3.1. Extraction Yield

Ethanolic extraction of *Capparis cartilaginea* leaves yielded 42.24 g of dried extract, corresponding to 4.69% (w/w) of the original dried plant material. The extract was dark green, indicating the presence of chlorophyll-rich and ethanol-soluble phytoconstituents (Table 1).

3.2. Phytochemical Screening

Qualitative phytochemical screening of the ethanolic leaf extract of *C. cartilaginea* revealed the presence of several biologically active secondary metabolites. The extract tested positive for carbohydrates, fixed oils, glycosides, anthraquinones, phenolic compounds (including tannins and flavonoids), saponins, and gum/mucilage. In contrast, alkaloids, phytosterols, and proteins were not detected in the samples (Table 2).

3.3. Effect of *C. cartilaginea* Extract on Acetic Acid–Induced Writhing Test

Administration of *C. cartilaginea* extract significantly reduced the number of acetic acid–induced writhing episodes in rats compared to that in the control group. The control group exhibited 18.20 ± 6.14 writhes, whereas treatment with the 200 mg/kg extract significantly reduced the number of writhes to 11.40 ± 2.07. A more pronounced antinociceptive effect was observed at 400 mg/kg, where the number of writhes was reduced to 5.60 ± 3.05. The standard drug diclofenac sodium (20 mg/kg) produced the greatest inhibitory effect, with a mean writhing count of 2.40 ± 1.14 (Table (3) and Figure (1)).

Table[1]: Percentage yield and physical characteristics of the ethanolic extract of *Capparis cartilaginea* leaves

Color of extract	Yield of extract (g)	Yield (%w\w)
Dark greenish	42.24	4.69

Table[2]: qualitative phytochemical screening of the ethanolic leaf extract of *Capparis cartilaginea*

Chemical test	Result
Alkaloids	-
Carbohydrate	+
Fixed oil	+
Glycoside	+
Anthraquinones	+
Phenolic compounds / Tannins / Flavonoids	+
Phytosterol	-
Protein	-
Saponin	+
Gum/Mucilage	+

+ = presence, - = absence

Table[3]: Effect of *Capparis cartilaginea* leaf extract and diclo-fenac on acetic acid–induced writhing in rats

Group	Mean ± SD	*P* Value
Control	18.20 ± 6.14	—
Cap. 200 mg	$11.40 \pm 2.07^{*\#}$	0.0060
Cap. 400 mg	$5.60 \pm 3.05^{*}$	0.0003
Diclofenac	$2.4 \pm 1.14^{*}$	< 0.0001

*significant different from control group $p < 0.05$, # significant different from diclofenac group $p < 0.05$, Cap 200 mg: *Capparis cartilaginea* leaf extract (200 mg/kg, i.p.), Cap 400 mg: *Capparis cartilaginea* leaf extract (400 mg/kg, i.p.),

4. DISCUSSION

The present study demonstrated that the ethanolic leaf extract of *Capparis cartilaginea* possesses significant dose-dependent peripheral antinociceptive activity in the acetic acid–induced writhing model, supporting its traditional use in pain-related conditions.

The extraction yield (42.24 g; 4.69% w/w) indicated

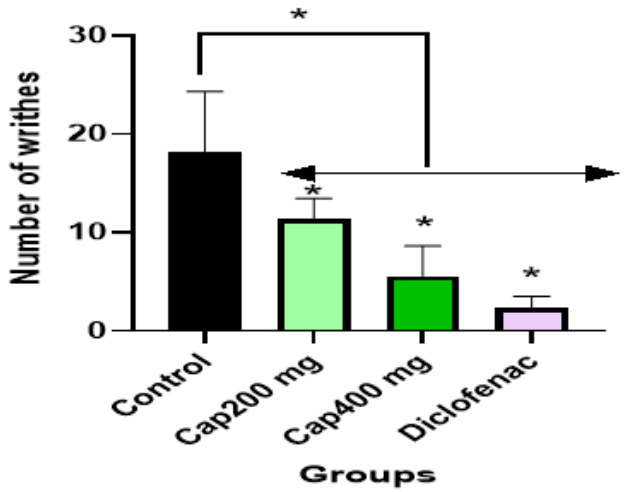

Figure 1. Effect of *C. cartilaginea* leaf extract (200 mg,400 mg /kg, i.p) on writhing test in rats. Values are expressed as Mean ± SD.

a satisfactory recovery of ethanol-soluble constituents. Ethanol is considered an effective extraction solvent for medicinal plants because of its ability to extract a broad range of bioactive polar and semipolar compounds, including flavonoids, tannins, glycosides, and other phenolic constituents with known pharmacological relevance [6, 11].

Preliminary phytochemical screening revealed the presence of several important secondary metabolites, including flavonoids, tannins, glycosides, saponins, anthraquinones, fixed oils, and gums/mucilage. These constituents are known to exhibit analgesic, anti-inflammatory, and antioxidant activities. In particular, flavonoids and tannins have been reported to suppress inflammatory pathways and nociceptive signaling by modulating prostaglandin synthesis, oxidative stress, and cytokine production [5, 7]. Similarly, saponins and glycosides may exert analgesic effects by inhibiting the production of inflammatory mediators [8, 15, 16].

The acetic acid–induced writhing model is a widely accepted method for evaluating peripheral analgesic activity, as it reflects nociceptive responses triggered by endogenous mediators, such as prostaglandins, bradykinin, histamine, serotonin, and inflammatory cytokines [13, 17]. In this study, the extract significantly reduced writhing responses in a dose-dependent manner, with the 400 mg/kg dose showing greater inhibitory activity than the 200 mg/kg dose and approaching the effect of diclofenac sodium. Since diclofenac acts primarily by inhibiting cyclooxygenase and prostaglandin biosynthesis, the observed effect of the extract may similarly involve the suppression of peripheral inflammatory mediators [3, 14].

These findings are consistent with previous studies on *Capparis* species, which have reported the presence of biologically active phenolic and flavonoid-rich constituents associated with antioxidant and anti-inflammatory activities that may underlie their analgesic potential [9–12, 18].

5. CONCLUSION

The present study demonstrated that the ethanolic leaf extract of *Capparis cartilaginea* possesses significant dose-dependent peripheral antinociceptive activity in the acetic acid–induced writhing model in rats. Preliminary phytochemical screening confirmed the presence of several bioactive secondary metabolites, particularly flavonoids, tannins, glycosides, saponins, anthraquinones, and other phenolic constituents, which may contribute to the observed analgesic effects.

The marked reduction in writhing responses, especially at the 400 mg/kg dose, suggests that the extract may exert its activity by inhibiting peripheral inflammatory mediators involved in nociception. These findings provide experimental pharmacological support for the traditional use of *C. cartilaginea* in pain management and indicate that this plant may be a promising natural source for developing safer analgesic agents.

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