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Antioxidant, antifungal and anti-inflammatory activities and acute toxicity of ethanol extracts from various organs of *Capparis spinosa* L.

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Abstract

Capparis spinosa L. is a versatile plant with prospects for biodiversity conservation, pharmaceutical applications and the food industry. The purpose of this study was to assess the composition, antioxidant activity, acute toxicity, anti-inflammatory activity and antifungal activity of extracts from various organs, as well as the total alkaloids and polyphenols of the plant *C. spinosa* growing in the Southern Aralkum. The antioxidant activity of *C. spinosa* organ extracts showed the highest activity in the stems-leaves [half-maximal inhibitory concentration (IC_{50}) of the lowest IC_{50} was 155 µg/mL], while the lowest activity was observed in the roots (with the highest IC_{50} of 535 µg/mL). The IC_{50} values for the total alkaloids and polyphenols were 124 and 182 µg/mL respectively. The median lethal dose (LD_{50}) of all the obtained substances was determined to be greater than 2000 mg/kg. Regarding anti-exudative effects, the reference drug indomethacin, at a dose of 10 mg/kg, showed 45.9 % inhibition, a level also observed with the stem-leaf and root extracts at doses of 100 mg/kg. The highest inhibition was exhibited by the total alkaloids at 200 mg/kg, demonstrating a 59.4 % anti-exudative effect. Among the tested pathogens, *Fusarium oxysporum* displayed the highest sensitivity to all the extracts. Specifically, the stem-leaf and root extracts effectively inhibited fungal growth (14 ± 1.2 mm). The fruit extract showed the strongest fungicidal property against *Phomosis* spp. (17 ± 1.1 mm). *Alternaria alternata* proved to be the most resistant strain to all tested preparations, with its growth inhibition zones ranging from 4–9 mm. In conclusion, the therapeutic efficiency and low toxicity of these *C. spinosa* extracts justify further development of this resilient plant as a source for natural functional foods and safe pharmacological applications.

Keywords: acute toxicity; anti-exudative; antifungal; antioxidant; *Capparis spinosa*; Southern Aralkum

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Introduction

Capparis spinosa L. is distributed across Southern Europe, Northern and Eastern Africa, Madagascar, Western, Central and Southeastern Asia, Australia and Oceania (1). Plant-derived medicinal products are considered unique therapeutic agents containing complex, multicomponent assemblies of biologically active substances. Herbal preparations are characterised by low toxicity, a broad therapeutic range, minimal side effects and relatively low cost. One such plant, *C. spinosa*, is widely utilised in traditional medicine for the treatment of various diseases. In folk medicine, the roots of *C. spinosa* are utilised as a diuretic, astringent and tonic. The root bark, characterised by its bitter taste, serves as an aperitif, astringent and stimulant agent and is employed in the treatment of diarrhoea, hemorrhoids and splenic diseases. Decoctions made from the stem and root bark exhibit antidiarrhoeal and antipyretic properties, while fresh fruits demonstrate positive therapeutic effects on edema and sciatica (2). Quantitative analysis of stachydrine distribution within *C. spinosa* organs demonstrated distinct tissue-specific accumulation. Significantly higher metabolite concentrations were recorded in the leaves (20.2 mg/g for Southern Italy and 32.3 mg/g for Saudi Arabia) compared to the roots (10.4 –12.5 mg/g) (3). The aqueous extract of *C. spinosa* has been shown to possess anti-edematous activity against

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carrageenan-induced paw edema in rats (4). The aqueous fruit extract of *C. spinosa* effectively inhibits carrageenan-induced paw edema in mice as well (5). *Capparis spinosa* exhibited anti-inflammatory activity by suppressing cytokine production in lipopolysaccharide -induced dendritic cells (6). An analysis of the phytochemical composition and antioxidant activity of *C. spinosa* leaf extracts collected from five regions of Southern Tunisia revealed that the maceration method exhibited the highest antioxidant activity (7). The half-maximal inhibitory concentration (IC_{50}) values for the 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity of the methanol and ethyl acetate extracts of *C. spinosa* were found to be 94.4 ± 4.5 and 57.75 ± 2.3 µg/mL respectively (8). The antioxidant compounds in the hydroethanolic extract of *C. spinosa* leaves, such as polyphenols, flavonoids and anthocyanins, demonstrated radical scavenging activity in DPPH and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. Additionally, the antioxidant effects of the hydroethanolic extracts on human cervix uteri epithelioid carcinoma cells exposed to Fe^{2+} or H_2O_2 were determined *in vitro* (9). The antimicrobial and antioxidant activities (DPPH, ABTS and ferric reducing antioxidant power (FRAP)) of *C. spinosa* leaves were evaluated due to the presence of lipophilic and hydrophilic compounds, including carotenoids, polysaccharides, amino acids, flavonoids and proanthocyanidins (10). *Capparis spinosa* has been reported to possess a wide range of biological activities, including antioxidant, anti-inflammatory, anticancer, antimicrobial and antidiabetic effects. When aqueous extracts of *C. spinosa* were orally administered to mice at 20 mg/kg, a reduction in blood glucose levels (antihyperglycaemic effect) and body weight gain in high-fat diet-fed mice was observed (11, 12). In studies conducted by Iranian researchers, 70 % hydroethanolic extracts of *C. spinosa* fruit led to a reduction in blood glucose levels and glycated haemoglobin (HbA1c) in patients with type 2 diabetes (13). The hydroethanolic extract of *C. spinosa* fruit significantly increased the number of pancreatic beta-cells, the diameter of the islets of Langerhans and insulin levels (14). The *in vitro* antifungal activity of various concentrations of *C. spinosa* ethanolic extracts was investigated against the fungi *Alternaria alternata*, *Fusarium oxysporum*, *Phoma destructiva*, *Rhizoctonia solani* and *Sclerotium rolfsii* (15). When different fractions of *C. spinosa* leaf extract were tested at a dose of 20 µL for their activity against the growth of the fungus, *Aspergillus niger*, the ethyl acetate fraction showed the best result with an inhibition rate of 12.71 %, while the diethyl ether and aqueous fractions demonstrated the lowest performance at 58.78 % and 58.01 % respectively (11).

The objectives of this study were: (1) to determine the acute toxicity of 70 % ethanol extracts, total alkaloids and polyphenols derived from the roots, stems-leaves, flowers and fruits of *C. spinosa* populations in the Southern Aral Sea region; (2) to evaluate the anti-inflammatory activity of these substances at doses of 100 and 200 mg/kg in comparison with the control and reference groups to identify the effective dosages; (3) to calculate the antioxidant activity and IC_{50} values of the plant extracts for free radical scavenging; (4) to determine the antifungal activity of the plant extracts.

The severe soil salinisation and degradation of the Southern Aralqum region, formed as a result of the Aral Sea desiccation, is exerting a profoundly negative impact on the productivity of both natural flora and agricultural crops. Establishing “Green Belts” represents the most effective strategy to mitigate this environmental crisis and prevent further desertification. In this context, investigating the biochemical adaptation mechanisms of *C. spinosa*, a species inherently adapted to high salinity and arid conditions, holds significant scientific and practical importance.

Materials and Methods

Samples of *C. spinosa* were collected between August and September 2024 from the Muynak district of the Republic of Karakalpakstan (Aralkum) at coordinates 43°77′43.3" N and 60°20′28.1" E. All samples were dried at room temperature in a shaded area, protected from direct sunlight and prepared for further analysis. The botanical identification and authentication of *C. spinosa* were performed by Dr. D M Tajidinova at the Institute of Botany, Academy of Sciences of Uzbekistan. A voucher specimen (IBuz. 00049) was deposited in the National Herbarium of Uzbekistan (TASH).

Preparation of ethanolic extracts

Samples of *C. spinosa* (roots, stems-leaves, flowers and fruits) were weighed at 10 g (exact amount) using an analytical balance and placed into a 500 mL flat-bottomed ground-glass flask. Then, 200 mL (exact volume) of 70 % ethanol solution was added to the samples. The extraction process was carried out using a magnetic stirrer (DLAB MS7-H550-S, China) at 35 °C for 2 hr. This process was repeated twice: the first extraction used 200 mL (exact volume) and the second used 100 mL (exact volume). After each extraction, the extract was filtered through filter paper and collected into a 1000 mL flat-bottomed flask. Subsequently, the ethanol was evaporated using a rotary evaporator (DLAB RE 100 S, China) at 50 °C and 60 rpm. The remaining aqueous portion was frozen with liquid nitrogen and dried using a lyophiliser (Labconco 720401050, USA).

Isolation of polyphenolic compounds

Crushed aerial parts of *C. spinosa* (100 g, 1 : 1 stem-to-leaf ratio, weighed on an electronic analytical balance (Aczet Pvt Ltd CY 224C, China)) were initially defatted with 200 mL of chloroform under magnetic stirring (DLAB MS7-H550-S, China) at 50–55 ℃ for 2 hr (3 cycles) to remove lipophilic pigments. The pre-treated plant residue was then extracted with 70 % ethanol at 50–55 ℃ for 3 hr (3 cycles). The combined ethanolic extracts were filtered and concentrated under vacuum using a rotary evaporator (DLAB RE 100 S, China). The resulting aqueous fraction was washed with chloroform (3 times, 200 mL) via liquid-liquid partitioning and the chloroform layer was discarded. The remaining aqueous phase was

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partitioned with ethyl acetate (3 times, 200 mL). The pooled ethyl acetate fractions were dried over anhydrous $MgSO_4$ for 24 hr at room temperature, filtered and evaporated under reduced pressure. The crude concentrate was precipitated with hexane, filtered, washed repeatedly with pure hexane and dried in a vacuum oven (BOV-V625F, Biobase Group, China) at room temperature to yield the total polyphenolic complex.

Isolation of alkaloid compounds

Total alkaloids were isolated from the plant samples following the method established in previous studies (16).

Liquid chromatography-mass spectrometry analysis

The analysis of the extracts was performed using an Agilent LC/MS/MS Q-TOF 6420B (USA) system under the following conditions: samples were dissolved in 0.1 % trifluoroacetic acid (TFA) and injected into the mass spectrometer using an Agilent Technologies 1260 Cap Pump. Aliquots of 2–5 µL were introduced via an Agilent Technologies Micro WPS autosampler onto a Zorbax SB C_{18} column, 5 µm (150 × 0.5 mm) at a flow rate of 15 µL/min. Mobile phase: A – 0.1 % solution of formic acid in water, B – acetonitrile + 0.1 % formic acid. Sorbed polyphenols were desorbed from the column at a flow rate of 15 µL/min using the following mobile phase gradient for solution B: 5 % for 3 min, 80 % for 25 –30 min and 5 % for 35 min. The mass spectrometry parameters were as follows: the ionisation source was electrospray (ESI); the drying gas (N_2) flow rate was 7 L/min at a temperature of 300 °C; the skimmer cone voltage was 65 V and the fragmentor voltage was 175 V. The mass detection range was 100–3000 m/z for MS mode and 50–2400 m/z for MS/MS mode. Ionisation was performed in both positive and negative modes.

Study of antioxidant activity

The antiradical activity of *C. spinosa* extracts (root, stem-leaf, flower, fruit, total alkaloids and polyphenols) was determined based on the scavenging of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical using a standard method. A 0.1 mM solution of the DPPH radical in ethanol produces a deep purple colour and a change in the optical density of the solution was observed upon the addition of the samples. The kinetics of the change in optical density were measured using a UV-5100 spectrophotometer (Metash, UV-5100, China) at a wavelength of 517 nm in a 3 mL cuvette with an optical path length of 1 cm. To compare the antiradical activity of the test samples, 50 µL of a 5 mg/mL concentration solution was used. For the quantitative assessment of the antiradical activity, the IC_{50} was employed, which represents the concentration of the test compound required to reduce the initial concentration of stable radicals by 50 % under steady-state reaction conditions (17).

Study of acute toxicity

The acute oral toxicity of the chemical substances was evaluated in accordance with the “fixed dose” procedure recommended by the Organisation for Economic Co-operation and Development (OECD) (18).

Acute toxicity study

The 70 % ethanol extracts, total alkaloids and polyphenols derived from the roots, stems-leaves, flowers and fruits of *C. spinosa* were administered orally at a single fixed dose of 2000 mg/kg to groups of same-sex animals in a stepwise procedure. The acute toxicity assessment was conducted on the 70 % ethanol extracts of each plant part as well as the total alkaloids and polyphenolic compounds. The experiments were performed on male outbred white laboratory mice with a body weight of 22 ± 2.0 g. The animals were housed under standardised vivarium conditions, with controlled environmental parameters including a relative humidity of 55–65 % and a temperature maintained at 22 ± 2 °C. Throughout the experimental period, the animals had *ad libitum* access to water and were fed a standard laboratory diet. Each experimental group consisted of five mice, with a total of 35 animals used in the study. All pharmacological tests were conducted on healthy, sexually mature mice following a 10–14 day quarantine period.

The experiment was conducted in two stages. In Stage I, two mice from each group were orally administered a single dose of 2000 mg/kg (based on the dry mass) of *C. spinosa* root, stem-leaf, flower and fruit 70 % ethanol extracts, as well as total alkaloids and polyphenolic compounds, in a volume of 0.4 mL via a specialised gastric gavage. The animals were monitored for 2–3 days and no mortality was recorded. In Stage II, the remaining three animals in each group received the same substances at the identical dose of 2000 mg/kg. Simultaneously, the control group was administered 0.4 mL of distilled water orally. During the first day of both stages, the general condition of the research animals, possible tremors and mortality were monitored hourly. Over the following two weeks, daily examinations were conducted to assess the general health, locomotor activity, skin and fur condition, respiration rate and depth, urination, body weight fluctuations and other physiological parameters of the animals across all groups. The animals were maintained on a standard diet with *ad libitum* access to food and water (19). At the conclusion of the experiment, the LD_{50} and the toxicity class of the *C. spinosa* extracts and isolated compounds were determined (20, 21). The obtained data were subjected to statistical analysis, with results expressed as the arithmetic mean (M) and the standard error of the mean (m). The probability level of $p < 0.05$ was considered to indicate statistically significant differences.

Study of anti-inflammatory activity

The classical “carrageenan-induced edema model” was employed for the preliminary assessment of anti-inflammatory activity (22). The study was conducted on 56 male outbred laboratory rats with a body weight of 190 ± 20 g. The animals were divided into 14 groups: one control group and 13 experimental groups, with four rats in each group (23). Inflammation was induced by injecting 0.1 mL of a 1 % carrageenan solution into the subplantar region of the rat’s hind paw (24). The test substances - including the 70 % ethanol extracts of *C. spinosa* roots, stems-leaves, flowers and fruits, as

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well as total alkaloids and polyphenolic compounds - were administered orally at two different doses (100 and 200 mg/kg) 1 hr prior to the carrageenan injection. Indomethacin (10 mg/kg) was used as a reference drug. The anti-inflammatory activity was assessed by measuring the paw edema volume at hourly intervals (1–4 hr) following the subplantar injection. The peak inflammatory response was recorded 3 hr after the carrageenan injection and the antiexudative activity was determined based on these results.

Study of antifungal activity

The antagonistic properties of *C. spinosa* ethanolic extracts were determined using the agar well diffusion method (25). The screening of the antifungal activity of the compounds was evaluated by measuring the diameter of the growth inhibition zone of the fungal test cultures in millimetres (mm). The study utilised test cultures of several fungi, including *Phomopsis* spp., *Fusarium solani*, *A. alternata* and *F. oxysporum*. These antifungal activity investigations were carried out in collaboration with scientists from the Laboratory of Unique Objects, Collection of Phytopathogens and Other Microorganisms at the Institute of Genetics and Plant Experimental Biology of the Academy of Sciences of the Republic of Uzbekistan. Specifically, 1 µL of the extract at a concentration of 1 mg/mL was introduced into each well. Fluconazole 10 mg/10 mL was employed as the positive control, while distilled water served as the negative control. After inoculation, the Petri dishes were incubated at 26 ± 1 °C for 48 hr before measuring the zones of inhibition.

Statistical analysis

For statistical analysis, GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA, USA) was employed. Results are expressed as mean ± standard deviation (SD). Statistical significance between the groups was determined by Student's *t*-test, with significance levels set at $p < 0.05$ and $p < 0.01$.

The IC_{50} values were calculated by non-linear regression analysis using OriginPro 2022 software (OriginLab Corporation, Northampton, MA, USA).

Results

Quantitative analysis of total plant extracts, alkaloids and phenolic compounds

The 70 % ethanol extracts of *C. spinosa* roots, stems-leaves, flowers and fruits were prepared and the total alkaloid content from the root portion, as well as the total polyphenol content from the stem-leaf portion were isolated and quantified. The obtained results are presented in Fig. 1.

The content of secondary metabolites in *C. spinosa* plants from the Southern Aralqum region increased significantly under the influence of salinity stress. Among the ethanolic extracts of *C. spinosa*, the fruit extract yielded the highest amount at 5.46 ± 0.1 g, while the root extract yielded the lowest at 1.96 ± 0.2 g.

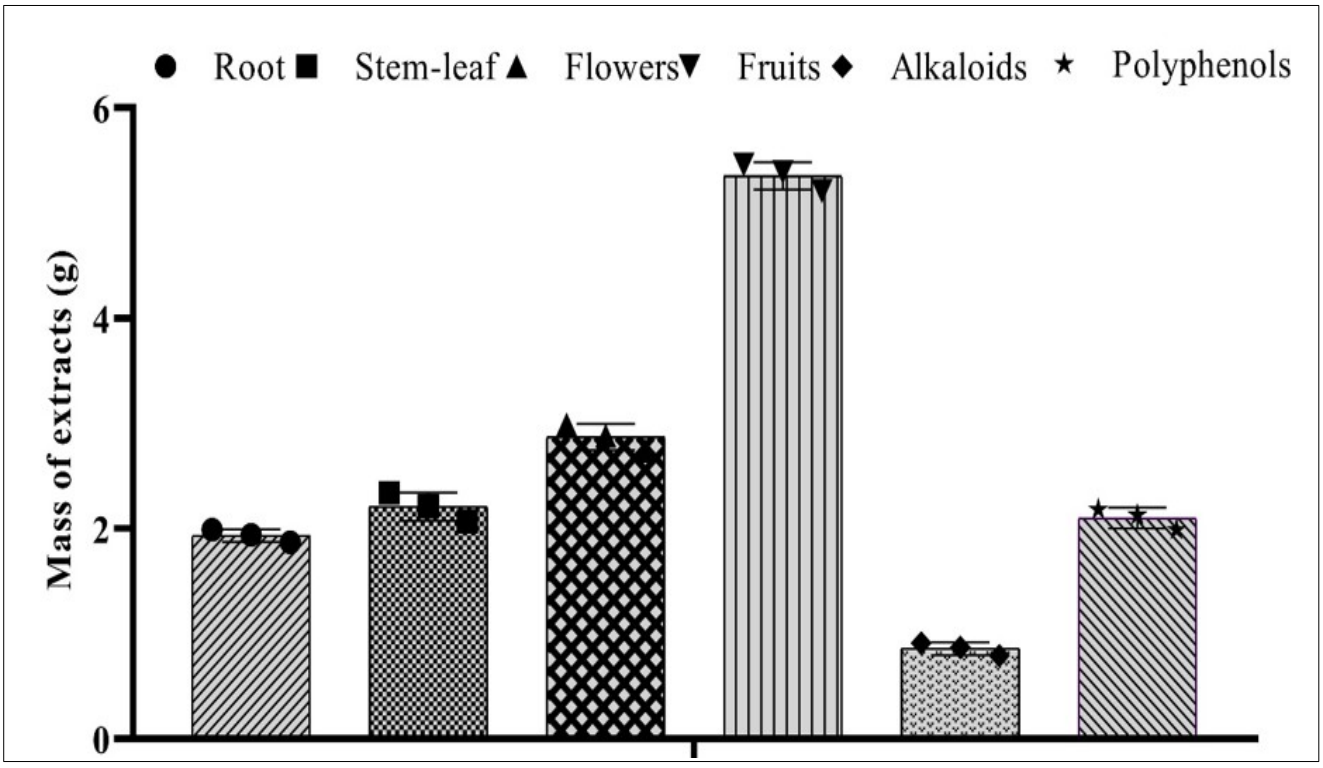


Fig. 1. The 70 % ethanol extracts of *Capparis spinosa* roots, stems-leaves, flowers and fruits were prepared and the total alkaloid content from the root portion as well as the total polyphenol content from the stem-leaf portion were isolated and quantified.

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Antioxidant activities

The IC_{50} values for the DPPH antioxidant activity of 70 % ethanol extracts from various organs of *C. spinosa*, as well as those of their total alkaloids and polyphenolic compounds, were calculated. The results are presented in Fig. 2.

According to the scientific literature, a lower IC_{50} value indicates greater antioxidant potential of a substance. The antioxidant capacity of *C. spinosa* organs decreased in the following order: stems-leaves > fruit > flower > root. The highest antioxidant activity was exhibited by the stem-leaf extract (IC_{50} = 155 µg/mL), suggesting a high concentration of bioactive compounds, particularly vitamins and flavonoids, in this part of the plant. Conversely, the root extract showed the lowest antioxidant activity (IC_{50} = 535 µg/mL). Furthermore, the total alkaloids isolated from the roots and the total polyphenols isolated from the stems-leaves yielded IC_{50} values of 124 and 182 µg/mL respectively. The fact that the crude stem-leaf extract exhibited higher activity than the isolated total polyphenols can be attributed to the synergistic effects between polyphenols and various other classes of antioxidants present in the extract (Table 1).

Acute toxicity of plant extracts, alkaloids and phenolic compounds

Upon oral administration of the 70 % ethanol extracts of *C. spinosa* roots, stems-leaves, flowers and fruits, as well as the total alkaloids and polyphenolic compounds at a dose of 2000 mg/kg, initial behavioural changes were observed in mice. Approximately 10 min post-administration, the animals exhibited tachypnea (increased respiratory rate), huddling behavior and ptosis (narrowing of the eyelids). These symptoms persisted for 20–30 min, after which the mice began to recover and returned to their normal physiological state. Throughout the entire observation period, no mortality was recorded (0/5) among the animals receiving the 2000 mg/kg dose of the dry extracts and isolated compounds.

No significant decrease in body weight was observed in the experimental groups on days 7 and 14 of the study compared to the control group ($p > 0.05$). Following a single oral administration of the 70 % ethanol extracts from the roots, stems-leaves, flowers and fruits of *C. spinosa*, as well as their total alkaloids and polyphenolic compounds at a dose of 2000 mg/kg, the median lethal dose was determined to be LD_{50} > 2000 mg/kg.

In the control group (untreated), the peak edema was observed at the 3 hr mark, reaching 78.0 ± 7.4 % relative to the baseline and remained elevated at 45.9 ± 4.4 % even after 4 hr. Similarly, the maximum swelling in the experimental groups was recorded 3 hr after the carrageenan injection; however, under the influence of the test compounds, the edema levels nearly returned to baseline values by the 4th hour. These hourly observations, expressed as percentages, are detailed in Table 2 and Fig. 3.

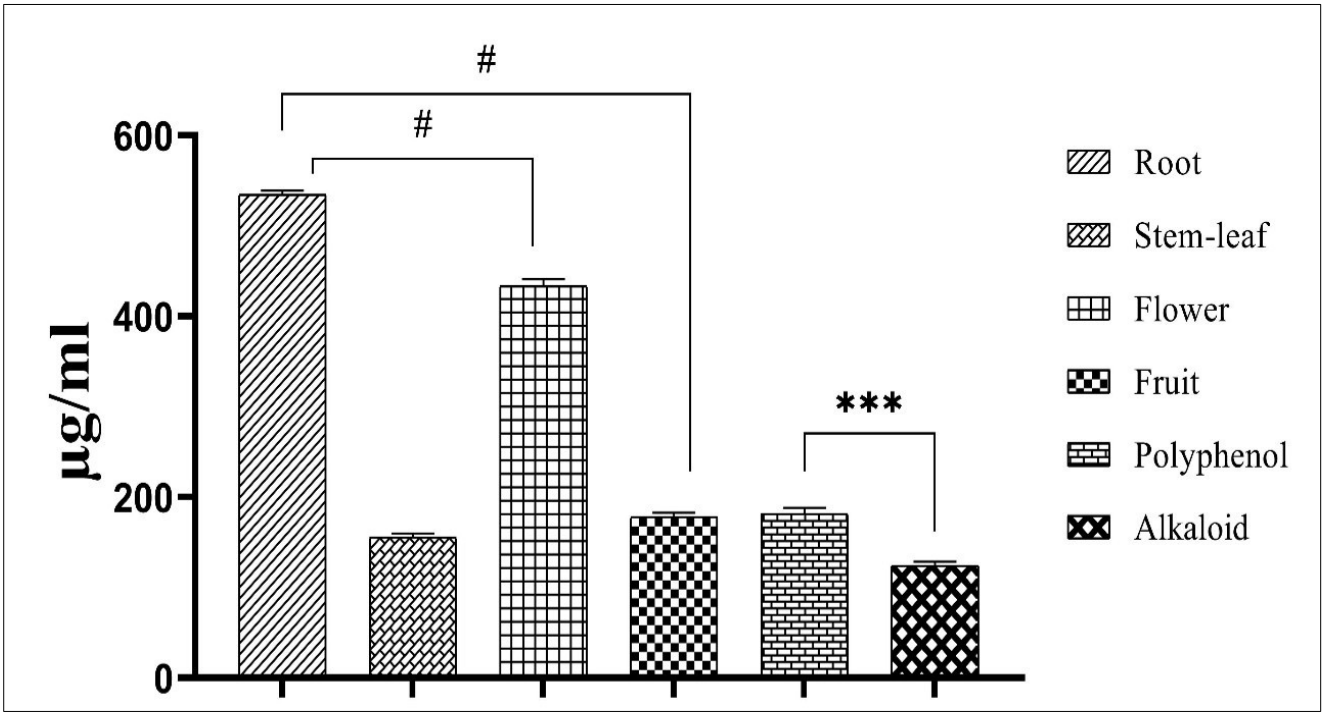


Fig. 2. The IC_{50} values for the DPPH antioxidant activity of 70 % ethanol extracts from various organs of *Capparis spinosa*, as well as their total alkaloids and polyphenolic compounds, were calculated. #$p < 0.0001$; ***$p < 0.0002$.

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Table 1. LC/MS/MS analysis results of the 70 % ethanol extracts from the roots, stems-leaves, flowers and fruits of *Capparis spinosa*, as well as total alkaloids from the roots and total polyphenols from the stems-leaves

Plant organs	No.	Compound	Brutto formula	Molecular weight	Identity
Root	1	Glucocapparin	$C_8H_{15}NO_9S_2$	333.30	332.03635 $[M+H]^-$
	2	Quinic acid	$C_7H_{12}O_6$	192.17	191.03334 $[M+H]^-$
	3	Neoglucobrassicin	$C_{17}H_{22}N_2O_{10}S_2$	478.50	477.09797 $[M+H]^-$
	4	Cappariloside B	$C_{22}H_{28}N_2O_{11}$	496.50	495.32405 $[M+H]^-$
Stem-leaf	1	Glucocapparin	$C_8H_{15}NO_9S_2$	333.30	332.03635 $[M+H]^-$
	2	Quinic acid	$C_7H_{12}O_6$	192.17	191.03334 $[M+H]^-$
	3	Neoglucobrassicin	$C_{17}H_{22}N_2O_{10}S_2$	478.50	477.09797 $[M+H]^-$
	4	Rutin	C27H30O16	610.50	609.19747 $[M+H]^-$
	5	Kaempferol 3-O-rhamnoside	$C_{27}H_{30}O_{15}$	594.50	593.21495 $[M+H]^-$
	6	Quercetin 3-O-rhamnoside	$C_{21}H_{20}O_{11}$	448.38	447.23343 $[M+H]^-$
	7	Caffeic acid	$C_9H_8O_4$	180.16	179.07357 $[M+H]^-$
	8	Syringic acid	$C_9H_{10}O_5$	198.17	197.80330 $[M+H]^-$
Flower	1	Quinic acid	$C_7H_{12}O_6$	192.17	191.03334 $[M+H]^-$
	2	Linalyl acetate	$C_{12}H_{20}O_2$	196.29	195.07178 $[M+H]^-$
	3	Quercetin 3-O-rhamnoside	$C_{21}H_{20}O_{11}$	448.38	447.23343 $[M+H]^-$
	4	Cirsiliol	$C_{17}H_{14}O_7$	330.29	329.26933 $[M+H]^-$
	5	α-Farnesene	$C_{15}H_{24}$	204.35	203.10246 $[M+H]^-$
	6	Kaempferol-3-O-rutinoside	$C_{27}H_{30}O_{15}$	594.50	593.21495 $[M+H]^-$
	7	Rutin	C27H30O16	610.50	609.19747 $[M+H]^-$
	8	Caffeic acid	$C_9H_8O_4$	180.16	179.07357 $[M+H]^-$
	9	Cadabicine	$C_{25}H_{29}N_3O_4$	435.50	434.15175 $[M+H]^-$
Fruit	1	Glucocapparin	$C_8H_{15}NO_9S_2$	333.30	332.03635 $[M+H]^-$
	2	Quinic acid	$C_7H_{12}O_6$	192.17	191.03334 $[M+H]^-$
	3	Caffeic acid	$C_9H_8O_4$	180.16	179.07357 $[M+H]^-$
	4	Linalyl acetate	$C_{12}H_{20}O_2$	196.29	195.07178 $[M+H]^-$
	5	Spinoside A	$C_{19}H_{30}O_9$	402.40	401.19071 $[M+H]^-$
	6	Cirsiliol	$C_{17}H_{14}O_7$	330.29	329.26933 $[M+H]^-$
	7	Quercetin 3-O-rhamnoside	$C_{21}H_{20}O_{11}$	448.38	447.23343 $[M+H]^-$
	8	Cadabicine	$C_{25}H_{29}N_3O_4$	435,50	434.15175 $[M+H]^-$
	9	Kaempferol 3-O-rhamnoside	$C_{27}H_{30}O_{15}$	594.50	593.22949 $[M+H]^-$
	10	Rutin	C27H30O16	610.50	609.19747 $[M+H]^-$
Total alkaloids isolated from the root	1	Stachydrine	$C_7H_{13}NO_2$	143.18	144.0400 $[M+H]^+$
	2	Capparispine	$C_{25}H_{29}N_3O_4$	435.50	436.23726 $[M+H]^+$
Total polyphenols are isolated from stem and leaf parts	1	Quinic acid	$C_7H_{12}O_6$	192.17	191.03334 $[M+H]^-$
	2	Rutin	$C_{27}H_{30}O_{16}$	610.50	609.19747 $[M+H]^-$

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Table 2. Evaluation of the acute toxicity parameters of 70 % ethanol extracts from the roots, stems-leaves, flowers and fruits of *Capparis spinosa*, as well as its total alkaloids and polyphenols, in male mice (M ± m, *n* = 5)

Samples	Dose (mg/kg mL)	Number of deaths/number of animals in the group	Average animal body weight (g)			LD_{50} (mg/kg)
			1 days	7 days	14 days	
Control	0.4 mL	5/0	22.3 ± 2.1	23.1 ± 2.3	24.3 ± 2.4	–
Root	2000 mg/kg	5/0	21.1 ± 2.1	22.3 ± 2.2	23.5 ± 2.3	>2000
Stem-leaf	2000 mg/kg	5/0	22.0 ± 1.9	22.4 ± 2.2	23.3 ± 2.3	>2000
Fruit	2000 mg/kg	5/0	20.5 ± 2.0	21.8 ± 2.2	22.3 ± 2.2	>2000
Flower	2000 mg/kg	5/0	21.0 ± 2.0	22.6 ± 2.2	23.8 ± 2.4	>2000
Total alkaloid	2000 mg/kg	5/0	21.4 ± 2.1	22.5 ± 2.2	23.7 ± 2.3	>2000
Total polyphenol	2000 mg/kg	5/0	22.1 ± 2.0	22.9 ± 2.2	23.8 ± 2.3	>2000

$p < 0.05$ compared to the control.

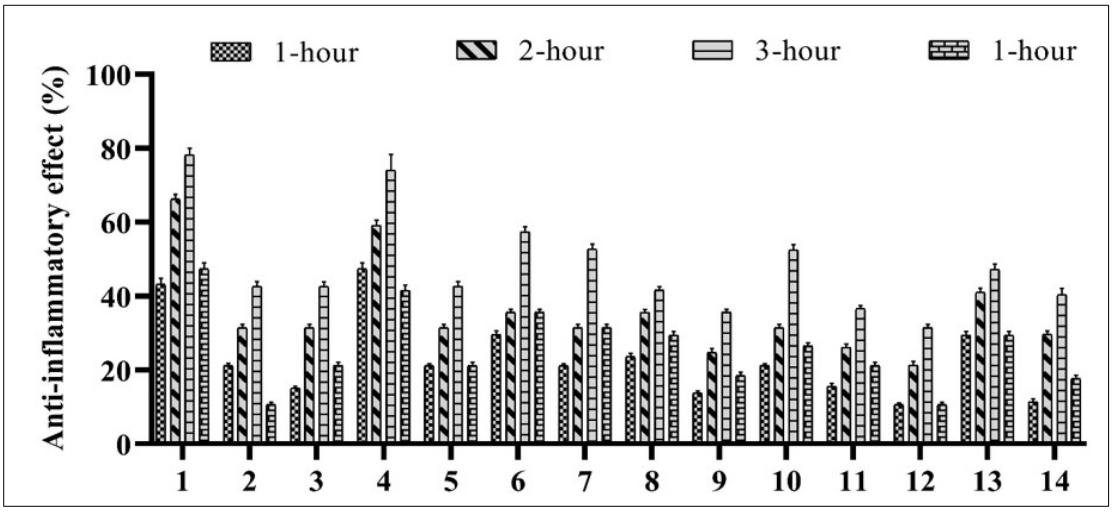


Fig. 3. Effects of 70 % ethanol extracts from the roots, stems-leaves, flowers and fruits of *Capparis spinosa*, as well as its total alkaloids and polyphenols, at two different doses on carrageenan-induced paw edema compared to indomethacin (expressed as % relative to the baseline; M ± m, *n* = 4). 1: Control (infected); 2: Indomethacin 10 mg/kg; 3: Root 100 mg/kg; 4: Root 200 mg/kg; 5: Stem-leaf 100 mg/kg; 6: Stem-leaf 200 mg/kg; 7: Fruit 100 mg/kg; 8: Fruit 200 mg/kg; 9: Flower 100 mg/kg; 10: Flower 200 mg/kg; 11: Total alkaloid 100 mg/kg; 12: Total alkaloid 200 mg/kg; 13: Total polyphenol 100 mg/kg; 14: Total polyphenol 200 mg/kg.

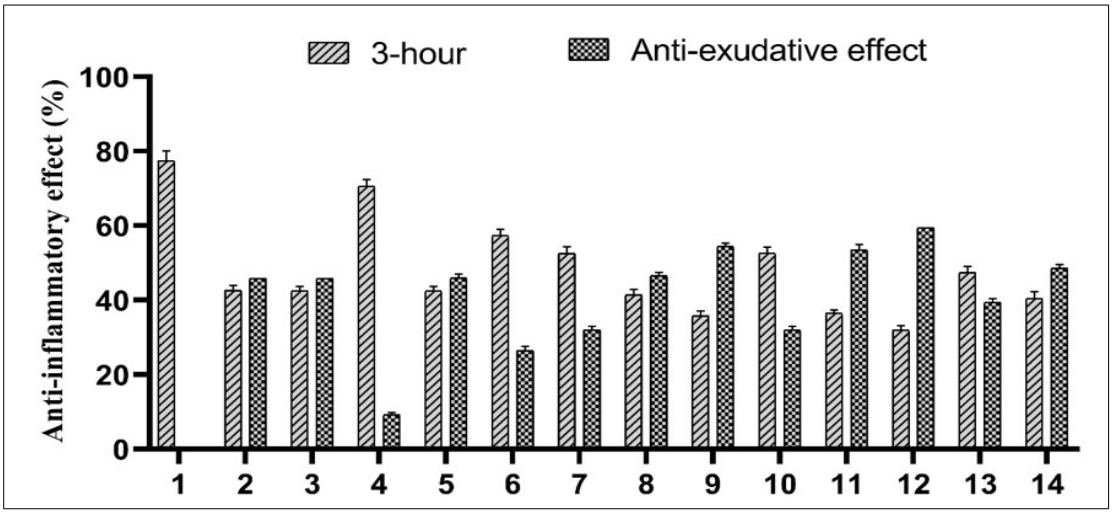


Fig. 4. Comparative anti-exudative activity of 70 % ethanol extracts from the roots, stems-leaves, flowers and fruits of *Capparis spinosa*, along with total alkaloids and total polyphenols at two different doses, relative to the control group and the reference drug (M ± m, *n* = 5). 1: Control (infected); 2: Indomethacin 10 mg/kg; 3: Root 100 mg/kg; 4: Root 200 mg/kg; 5: Stem-leaf 100 mg/kg; 6: Stem-leaf 200 mg/kg; 7: Fruit 100 mg/kg; 8: Fruit 200 mg/kg; 9: Flower 100 mg/kg; 10: Flower 200 mg/kg; 11: Total alkaloid 100 mg/kg; 12: Total alkaloid 200 mg/kg; 13: Total polyphenol 100 mg/kg; 14: Total polyphenol 200 mg/kg.

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Anti-inflammatory activities

Based on the data, all the studied substances exhibited anti-exudative effects. The reference drug, indomethacin (10 mg/kg), showed a remaining edema level of 45.9 % at the 4 hr mark following carrageenan administration. Notably, the anti-exudative activities of the stem-leaf and root extracts at a dose of 100 mg/kg did not significantly differ from the reference drug, also yielding values around the 45.9 % range. For the remaining samples, the anti-exudative effects at doses of 100 and 200 mg/kg varied between 9.2 % and 59.4 %. Although the peak edema in the experimental groups was observed 3 hr post-carrageenan injection, the anti-exudative efficacy was calculated relative to this 3 hr peak. The results of this percentage-based inhibition are presented in Fig. 4.

The anti-exudative activity (AEA) was calculated using the following formula:

$$AEF = [(\Delta V_n - \Delta V_{taj})/\Delta V_n] \times 100\ \%$$

Where, AEF: anti-exudative activity, expressed as a percentage; ΔV_n: the change in hind paw volume of the control group; ΔV_{taj}: the change in hind paw volume of the experimental group.

Antifungal activities

Antifungal activity was determined by assessing whether the alcoholic extracts, total alkaloids and polyphenols isolated from *C. spinosa* inhibited the growth and development of fungi such as *Phomopsis* spp., *F. solani*, *A. alternata* and *F. oxysporum*. The results of these inhibitory effects are presented in Fig. 5.

The results reflect the antifungal activity of various extracts and fractions (alkaloids, polyphenols) from *C. spinosa* against phytopathogenic fungi are presented in this study. The data indicates that the plant extracts exhibited varying degrees of selective activity against all tested phytopathogens. The absence of an inhibition zone (0 mm) in the negative control group (sterile water) confirmed the validity of the experimental conditions; however, the lack of an inhibition zone in the positive control group (fluconazole) indicated that the tested fungal strain was resistant to the reference drug. *Fusarium oxysporum* displayed the highest sensitivity to all tested extracts, with the stem-leaf and root extracts showing particularly effective inhibition (14 ± 1.2 mm). Against *Phomopsis* spp., the fruit extract exhibited the most potent fungicidal properties (17 ± 1.1 mm). Conversely, *A. alternata* proved to be the most resistant strain among all tested preparations, with inhibition zones limited to a range of 4–9 mm. These findings suggest that the alcoholic extracts of *C. spinosa* contain bioactive components that adversely affect fungal development, warranting further investigation.

Discussion

The concentration of phytochemicals within the extracts varied depending on the extraction method employed (7). The observed increase in phenolic compounds and flavonoid content under the high salinity conditions of the Southern Aralqum serves to enhance the antioxidant potential of the plant. This, in turn, protects cellular membranes from oxidative damage, thereby ensuring the survival and sustainable growth of the species within such an extreme environment (26). The contents of rutin and quercetin in the *C. spinosa* plant were found to be 1.02 and 6.3 mg/g in the roots, 1.95 and 8.82 mg/g in the stems, 10.4 and 25.82 mg/g in the leaves and 9.4 and 11.7 mg/g in the flower buds respectively (27). Chinese researchers identified seven primary compounds in the ethanol : water (50 : 50) extract of *C. spinosa* fruit: p-hydroxybenzoic acid, 5-(hydroxymethyl) furfural, bis(5-formylfurfuryl) ether, daucosterol, methyl ester of alpha-D-fructofuranoside, uracil and stachydrine (28). The extracts of *C. spinosa* flower buds contained glucocapparin (2.05 ± 0.25 mg/g) as the most abundant glucosinolate (GLS), followed by glucobrassicin (232 ± 18 µg/g) and 4-hydroxyglucobrassicin (89 ± 12 µg/g) (29).

Previous studies have reported that the stem and leaf parts of *C. spinosa* possess significant antioxidant potential. The IC_{50} values for DPPH radical scavenging were 7.4 µg/mL for the leaf extract and 10.2 µg/mL for the stem extract. These findings indicate higher antioxidant efficacy of bioactive compounds in the leaves compared to the stems (30). Previous research has also demonstrated distinct radical scavenging activity of methanolic *C. spinosa* extracts against DPPH. As the extract concentration increased from 31.25–1000 µg/mL, the inhibition rate of free radicals increased proportionally from 7.952–79.142 %. The IC_{50} for this extract was determined to be 440.237 µg/mL (31). Studies on the DPPH activity of methanolic extracts from *C. spinosa* leaves collected from various regions have revealed distinct geographical variations. Specifically, samples from the Skuru region exhibited the highest antioxidant activity, with an IC_{50} value of 31.0 µg/mL. These findings confirm that the biosynthesis and accumulation of bioactive compounds in the plant vary significantly depending on eco-geographical factors (32). Previous studies have reported that the antioxidant activity of *C. spinosa* varies significantly depending on both the plant organs and their respective habitats. Across all investigated regions, the fruits exhibited the highest antioxidant potential (15–16 %), which was attributed to the elevated concentration of flavonoids and other phenolic compounds within these reproductive structures. Conversely, the lowest activity was observed in the roots, ranging between 3 % and 4 %). On a regional basis, the higher values recorded in certain organs from Saravan and Iranshahr samples indicate that localised abiotic stressors in these areas may activate the plant's antioxidant defence systems (33).

When the safety of the polyphenolic complex isolated from *C. spinosa* L. leaves was tested in laboratory animals, all studied doses (25–100 mg/kg) caused no mortality. Previous research concluded that this extract was free of adverse toxic effects when administered at therapeutic doses (34).

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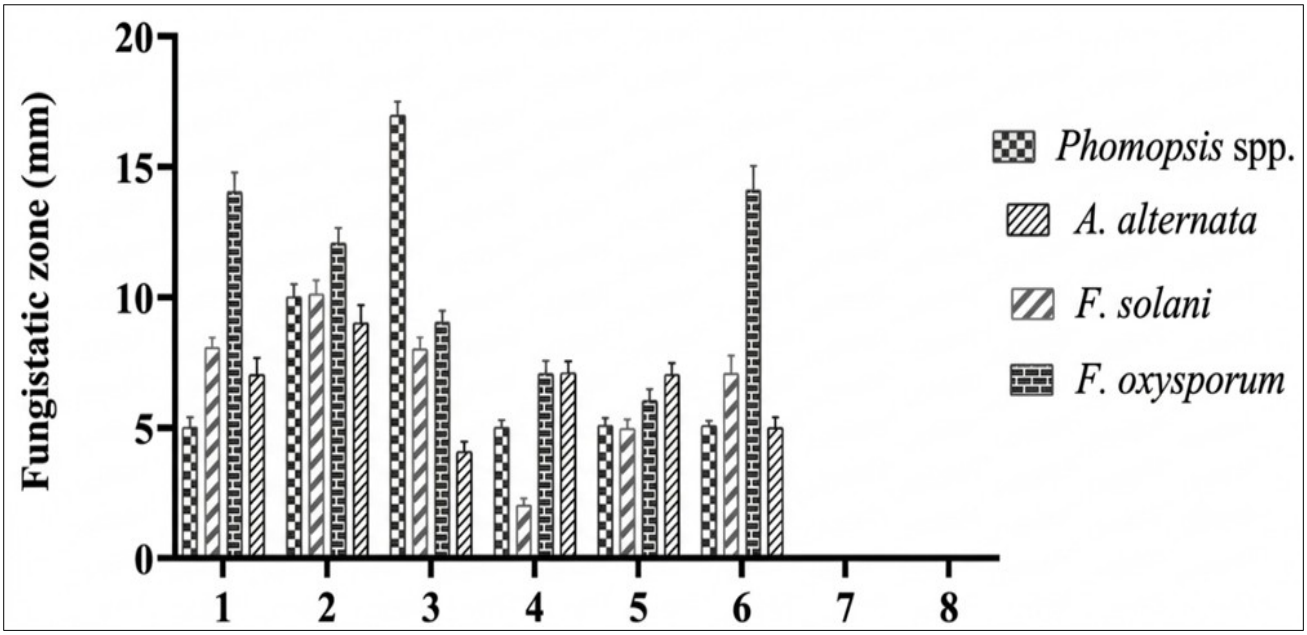


Fig. 5. Antifungal activity of the alcoholic extracts, total alkaloids and polyphenols isolated from *Capparis spinosa* against various fungal strains (M ± m, *n* = 5). 1: Stem-leaf; 2: Flower; 3: Fruit; 4: Total alkaloid; 5: Total polyphenol; 6: Root; 7: Control (positive); 8: Control (negative).

Previous studies have reported that the aqueous extract of *C. spinosa* fruit at doses of 50 and 250 mg/kg failed to inhibit carrageenan-induced inflammation in mice when observed 3 hr post-injection. However, the 50 % and 70 % ethanol extracts at a dose of 250 mg/kg demonstrated inhibitory effects of 40.8 % and 39.3 % respectively (35).

In an *in vitro* study investigating the antifungal activity of *C. spinosa* root ethanolic extract at concentrations of 0 %, 3 %, 6 % and 9 % (v/v), the highest efficacy was observed against *P. destructiva*, which exhibited a 9 cm zone of inhibition at all tested concentrations (3 %, 6 % and 9 %). Conversely, the lowest activity was recorded against *A. alternata*, with inhibition zones of 2.2, 1.7 and 1.5 cm respectively (15). Furthermore, when the effects of *C. spinosa* ethanolic extracts at concentrations of 250, 500 and 750 mg/100 ml were studied against the fungi *R. solani*, *Macrophomina phaseolina*, *Fusarium graminearum* and *A. alternata*, it was reported that the extract exhibited antifungal activities of 47.25 %, 67.20 % and 73.80 % respectively, against *A. alternate* (36). The antifungal efficacy of several plant essential oils against the fungus *A. alternate* was tested under laboratory conditions (*in vitro*). According to the results obtained, among the test groups, *Pongamia pinnata* essential oil inhibited the mycelial growth and spore germination of the pathogen to the highest degree, demonstrating that it is a potent natural agent against this rot disease (37). The obtained results confirm that fluconazole, a medical antifungal agent belonging to the triazole group, exhibits a limited spectrum of activity or lacks significant fungistatic efficacy against phytopathogenic fungi such as *Phomopsis* spp., *F. solani*, *F. oxysporum* and *A. alternata*. It is well established that fluconazole exerts its fungistatic effect by inhibiting lanosterol 14α-demethylase (CYP51), a key enzyme in the biosynthesis of ergosterol, an essential component of the fungal cell membrane. Nevertheless, these phytopathogenic species demonstrate a high degree of intrinsic resistance to this compound (38). Species belonging to the genus *Fusarium* display extremely high minimum inhibitory concentration (MIC) values for fluconazole. This phenomenon is primarily attributed to drug efflux pumps that actively expel molecules from the cell, structural variations in the *CYP51* gene and robust antioxidant enzyme systems that mitigate oxidative stress (39). Furthermore, the cell wall architecture and detoxification mechanisms of plant pathogens such as *A. alternata* and *Phomopsis* spp. are adapted to withstand medical azoles, explaining the lack of efficacy in suppressing their growth *in vitro* (40).

Study limitations

Our study has several limitations that should be considered when interpreting the results. First, the biological activities were evaluated using crude ethanolic extracts and total alkaloid and polyphenol fractions, while the specific compounds responsible for the observed antioxidant, anti-inflammatory and antifungal effects were not isolated and individually tested. Second, the toxicity assessment was limited to acute oral toxicity and no sub-chronic or chronic toxicity studies were conducted. Third, the anti-inflammatory activity was assessed using a single carrageenan-induced edema model with a relatively small number of experimental animals and the underlying molecular mechanisms were not investigated. Finally, the antifungal activity was evaluated only under *in vitro* conditions against a limited number of phytopathogenic fungi; therefore, in future studies, we will evaluate efficacy under field conditions and establish the mechanisms of antifungal action.

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Conclusion

The antioxidant potential of the 70 % ethanol extracts from various organs of *Capparis spinosa* followed the decreasing order: stems-leaves > fruit > flower > root. Toxicity studies confirmed that the median lethal dose for all extracts, total alkaloids and polyphenols was LD_{50} > 2000 mg/kg, classifying them as low-toxicity substances. Among the fractions, the total alkaloids exhibited the most potent anti-exudative activity. Furthermore, the antifungal efficacy was organ-specific: the fruit extract most effectively inhibited the growth of *Phomopsis* spp., while the stem-leaf and root extracts demonstrated significant inhibitory effects against *Fusarium oxysporum*.

Future research should focus on isolating and characterising the specific individual alkaloid and phenolic compounds responsible for these observed bioactivities. Additionally, *in vivo* studies evaluating their specific molecular mechanisms and long-term chronic toxicity studies are warranted to fully establish their clinical safety and therapeutic efficacy.

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Authors' contributions

ZJ designed the study. QK, ML, RD and GU conducted the experiment and analysed the data. AG, OS, IU and BN prepared the manuscript. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

All preoperative and experimental protocols were reviewed and formally approved by the Institutional Animal Care and Use Committee. All experimental procedures and animal handling practices were carried out in strict compliance with the European Directive 2010/63/EU, which governs the ethical use of animals in scientific research and ensures adherence to internationally accepted standards of animal welfare.

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