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Anti-angiogenic and Antioxidant Activities of *Capparis spinosa* L. in Rat Aortic Ring and CAM Models

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ABSTRACT
Objective: Angiogenesis is the formation of new blood vessels from pre-existing vasculature, a basic and tightly controlled biological process. Angiogenesis is essential for tissue regeneration, wound healing, and embryonic development under normal physiological conditions. However, under pathological conditions, dysregulated angiogenesis contributes to several diseases, including cancer, making it an important therapeutic target. Accordingly, natural products, including *Capparis spinosa* L. have attracted considerable attention as promising anti-angiogenic agents. *C. spinosa* is rich in bioactive phytochemicals such as flavonoids and phenolic compounds that have been reported to possess anti-angiogenic potential. This study aimed to evaluate the anti-angiogenic and antioxidant activities of *Capparis spinosa* leaf extracts using complementary *ex vivo* and in *vivo models*.
Methods: The rat aortic ring anti-angiogenesis assay, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, and chick chorioallantoic membrane (CAM) assay were carried out in the tissue culture laboratory of the Department of Pharmacology, College of Pharmacy, Al-Nahrain University.
Results: The ethanolic extract demonstrated significant inhibition of microvessel outgrowth in the rat aortic ring assay, with an IC_{50} value of 16.2 µg/mL and reduced neovascularisation in the CAM model, with an inhibition zone of 12±1.83 mm. The extract also exhibited concentration-dependent antioxidant activity in the DPPH assay.
Conclusions: The ethanolic extract of *Capparis spinosa* L. exhibits significant anti-angiogenic and antioxidant activities and represents a promising natural source of compounds capable of modulating angiogenesis and oxidative stress, supporting its potential therapeutic applications.
Keywords: *Capparis spinosa*; Anti-Angiogenic activity; Antioxidant activity; Rat aortic ring; CAM assay.

INTRODUCTION

The vascular system is essential for maintaining tissue homeostasis through the delivery of oxygen and nutrients and the removal of metabolic waste. Blood vessels are composed of endothelial cells supported by smooth muscle cells and pericytes. These cells collaborate to regulate the tone, permeability, and tissue perfusion of blood arteries [1, 2]. New blood vessel formation occurs primarily through two mechanisms: vasculogenesis, which predominates during embryonic development, and angiogenesis, when new capillaries arise from pre-existing vessels. Angiogenesis is a tightly regulated process that occurs in multiple steps. These processes include the activation of endothelial cells, degradation of the basement membrane, migration, proliferation, tube formation, and stabilisation of blood vessels [3, 4].

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This process is controlled by a balance between pro-angiogenic and anti-angiogenic factors. Among the most important regulators are vascular endothelial growth factor (VEGF), hypoxia-inducible factor-1α (HIF-1α), matrix metalloproteinases (MMPs), platelet-derived growth factor (PDGF), and inflammatory mediators such as tumour necrosis factor (TNF) [5, 6].

The disruption of this balance contributes to pathological angiogenesis, particularly in cancer, as neovascularisation supplies tumours with essential nutrients and oxygen, facilitating their growth and metastasis [7].

Aberrant angiogenesis is implicated in the progression and pathology of various diseases including diabetic retinopathy, autoimmune diseases, rheumatoid arthritis and atherosclerosis, where excessive vascular growth fuels tissue damage and chronic inflammation [8].

Numerous anti-angiogenic medicines that inhibit VEGF and related pathways are presently employed in clinical practice. Nonetheless, their efficacy is frequently constrained by resistance mechanisms, compensatory signalling pathways, and adverse effects. Identifying safer and more efficacious angiogenesis-modulating drugs remains a critical research focus. Despite the availability of synthetic anti-angiogenic medications, there is an increasing necessity to explore alternative bioactive molecules, particularly those derived from medicinal plants, which may offer multi-target mechanisms and reduced side effects. However, many traditionally used plants have not been sufficiently investigated for their potential role in regulating angiogenesis [9][10].

Capparis spinosa L., a medicinal plant widely distributed in the Mediterranean region, and traditionally used for inflammatory and hepatic disorders, has attracted increasing scientific interest due to its rich content of bioactive secondary metabolites, including flavonoids and phenolic compounds [11].

Capparis spinosa L. is known for its rich phytochemical composition, particularly flavonoids and phenolic compounds, which are associated with antioxidant and anti-angiogenic activities [12]. In the present study, the leaves of Capparis spinosa L. were selected due to their high content of bioactive compounds, particularly flavonoids and phenolic constituents [13]. These compounds may influence angiogenesis by modulation of oxidative stress and inhibition of pro-angiogenic signalling pathways such as VEGF [14]. However, despite these promising properties, the anti-angiogenic potential of *Capparis spinosa* has not been fully explored using combined experimental models. It is hypothesized that the bioactive compounds present in *Capparis spinosa* may exert anti-angiogenic and antioxidant effects through modulation of VEGF signalling and oxidative stress pathways. Therefore, this study aimed to evaluate its biological activity using complementary ex vivo and in vivo approaches.

MATERIALS AND METHODS

Materials

The study was conducted between October 2025 and February 2026. The rat aortic ring anti-angiogenesis assay, the DPPH radical scavenging assay, and the chick chorioallantoic membrane (CAM) assay were performed in the Tissue Culture Lab at the Department of Pharmacology, College of Pharmacy, Al-Nahrain University, Baghdad, Iraq.

Chemicals and reagents

L Glutamine (Sigma-Aldrich); Serum-free medium M199 solution (Sigma-Aldrich); Thrombin 100 IU vial (Sigma-Aldrich, USA). Ethanol, chloroform, Dimethyl sulfoxide (DMSO) and DPPH solution and the other reagents required for biochemical analyses were obtained from standard commercial suppliers. All chemical and reagents used were of analytical grade.

Plant collection and authentication

In July 2025, fresh aerial parts of *Capparis spinosa* L. were picked from wild plants in Al-Musayyib city, Babil Governorate, Iraq. The plant was taxonomically authenticated at Al-Razi center for alternative medicine, Ministry of Health, Iraq, and as *Capparis spinosa* L.(Family: Capparaceae). An official authentication certificate (No. 441, dated 7 January 2025) was obtained.

Plant Preparation and Extraction

The collected leaves were cleaned, air-dried at room temperature until constant weight, pulverized to a fine powder, and stored in an airtight glass container containing silica gel desiccant until use. In this study, three solvents with different polarities were selected for extraction on 130 g of dried *Capparis spinosa* leaf powder: chloroform, 70% ethanol, and distilled water. Different solvents of varying polarity were used to ensure the extraction of a wide range of phytochemical constituents. The extraction was carried out using a Soxhlet apparatus for 6 hours until complete extraction was achieved. After the extraction process, the solvent was evaporated under reduced pressure to recover the crude extract, the residues were collected and weighed. The extraction yield was then calculated as a percentage of the original weight of the dried plant material by this equation [15]:

Extraction yield (%) = (weight of dried extract /initial weight of plant material) × 100

Anti-Angiogenic Assessment via the Ex Vivo Rat Aortic Ring Model

The antiangiogenic effect was assessed using the ex vivo rat aortic ring model according to the protocol with slight procedural adjustment [16]. The adult male albino rats (12–14 weeks old) were anesthetized with diethyl ether and sacrificed in accordance with the institution's ethical guidelines.

The thoracic aorta was aseptically excised and promptly immersed in Hank's Balanced Salt Solution (HBSS), and carefully cleaned of surrounding connective and adipose tissues. After that, the aorta was sectioned into approximately 1 mm thick rings. Each aortic ring was placed on a 48-well tissue culture plate. Each well was supplemented with 300 µL of M199 medium containing 3 mg/mL of fibrinogen and 5 µg/mL of aprotinin. The addition of 10 µL of thrombin solution (50 NIH U/mL) to each well started the process of forming fibrin clots. Plates were incubated at 37°C in a humidified 5% CO_2 atmosphere for 15 minutes to allow complete gel polymerization.

After gel polymerization, a top layer of 300 µL M199 medium was added to each well. This layer was made up of 20% heat-inactivated fetal bovine serum (HIFBS), 0.1% ε-aminocaproic acid, 1% L-glutamine, and 0.6% gentamicin.

Stock solutions were prepared of chloroform, ethanolic, and aqueous extracts of *Capparis spinosa* L. in DMSO at a concentration of 10 mg/mL. The stock solutions were diluted with culture medium to reach a final working concentration of 100 µg/mL for biological testing. The extract was added to the upper layer medium immediately after the fibrin clot formed, making sure that the final DMSO concentration did not exceed 1% (v/v). A 1% DMSO-treated group was included as the negative control, while suramin (100 µM) was used as the reference positive control.

The length of the newly formed microvessels was quantified by using ImageJ software (NIH, USA) for analysis of standardized digital images. To determine the extent of angiogenesis inhibition, the percentage reduction was determined using the following formula:

Inhibition (%) = (1 - (At / Ac)) × 100

At indicates the average radial microvessel growth distance in the treated group (in mm), and Ac represents the corresponding growth distance in the negative control group. Each experimental condition was conducted in triplicate (n = 3). The obtained results were expressed as a percentage of inhibition ± SD.

Chick Chorioallantoic Membrane (CAM) Assay

The angiogenic potential of the ethanolic extract of *Capparis spinosa* L. was investigated using (CAM) assay [17]. Fertilized chicken eggs were obtained from a hatchery in Baghdad, Iraq. Prior to incubation, the outer shell surface was carefully sterilized using 70% ethanol to remove possible microbial contamination. Eggs were maintained in an incubator set at 37 °C with approximately 60% humidity.

Following 72 h of incubation, nearly 2 ml of albumin was gently withdrawn from the blunt end of each egg using a sterile syringe. After that, the incision was closed to relieve internal pressure and help the CAM separate from the shell. After that, the eggs were put back in the incubator for another 24 hours.

On day four, a circular window approximately 2–3 cm in diameter was aseptically created in the eggshell to expose the chorioallantoic membrane (CAM). Before treatment, the exposed CAM was visually checked to make sure that the blood vessels were still intact. A stock solution (10 mg/mL) of *Capparis spinosa* L. extract was first prepared in dimethyl sulfoxide (DMSO) and subsequently diluted to obtain the desired working concentration. After that, sterile paper discs were soaked in 50 µL of the prepared extract and allowed to dry in a sterile environment. After that, the modified filter paper discs were carefully placed on the CAM surface. After that, sterile adhesive tape was used to seal the window, and the eggs were put back in the incubator so that the angiogenic response could be measured again under the same controlled conditions.

Images were digitally analyzed to quantify the extent of vascular inhibition. This was done by measuring the distance (in mm) from the disc to the nearest visible blood vessel, indicating inhibition of angiogenesis. Responses

were classified into three groups: + (3–6 mm), ++ (6–9 mm), and +++ (> 10 mm). The results were given as mean ± standard deviation.

Antioxidant Assay

The DPPH free radical scavenging assay was used to evaluate the antioxidant activity of ethanolic extract of *Capparis spinosa* L.
A fresh 0.1 mM DPPH solution was prepared for by mixing 39.4 mg of DPPH with 1 L of methanol in an amber volumetric flask. The mixture was gently agitated until a clear homogenous solution was obtained. The prepared solution was kept in the dark to minimize light-induced degradation.
A stock solution of *Capparis spinosa* L. extract was prepared by mixing 100 µl of the extract with 990 µl of methanol. The final volume was 1 ml. A serial dilutions were prepared to make concentrations of 100, 50, 25, 12.5, 6.25, and 3.125 µg/mL.
All reactions were carried out in 96-well microplates under controlled laboratory conditions, where 200 µL of the DPPH solution was mixed with 100 µL of each extract concentration. Methanol served as the blank, and methanol with the DPPH solution was the negative control. Each concentration was tested in three independent replicates. After that, the plates were gently mixed and left at room temperature for 30 minutes, out of direct sunlight.
After incubation, an ELISA microplate reader measured absorbance at 517 nm. Lower absorbance values reflected increased free radical neutralization by the extract.
The percentage of DPPH radical scavenging activity was determined using the formula described by Adebiyi et al. [18]:

DPPH Scavenging Activity (%) = [(A control – A sample) / A control] × 100

In this formula:
- A_control is the absorbance of the DPPH solution without any extract
- A_sample is the absorbance of the extract after it has reacted with DPPH

Statistical Analysis

Results were expressed as mean ± standard deviation (SD). Differences were considered statistically significant at $P < 0.05$. IC_{50} values were calculated using linear and logarithmic regression analysis. All statistical analyses were carried out using SPSS software (version 21.0). All experiments were conducted in triplicate. Differences between groups were assessed using one-way analysis of variance ANOVA followed by Tukey's post-hoc test for multiple comparisons. [19].

RESULTS

Extraction process

The ethanolic extract had the highest extraction yield. indicating its superior capacity to extract bioactive constituents from *Capparis spinosa* L., as shown in Table 1.

Table 1: Weight and yield percentage obtained from *Capparis spinosa* leaf powder extracts.

Type of extract	Weight of crude extract	Yield %
Chloroform	2.10 g	1.62%
Ethanol	3.00 g	2.30%
Water	2.60 g	2.00%

Anti-angiogenic Activity of *Capparis spinosa* L. Leaf extracts evaluated using the rat aorta ring assay

Table 2, Figure 1 show that the ethanolic extract exhibited the highest inhibition of microvessel outgrowth (58.60%), followed by the aqueous extract (17.0%), whereas the chloroform extract demonstrated the lowest inhibitory activity (12.50%). Suramin (100 µg/mL), used as a positive control, produced 79% inhibition of angiogenesis. In contrast, the negative control (1% DMSO) showed no observable inhibition.
Figure 2 shows representative microscopic images that support these results. Microscopic examination confirmed a marked reduction in microvessel sprouting in aortic rings treated with ethanolic extract compared with the aqueous- and chloroform- treated groups. Statistical analysis revealed a significant difference ($P < 0.05$) between the experimental groups and the negative control.

Table 2 : Percentage inhibition of microvessel growth induced by chloroform, ethanol, and aqueous leaf extracts of *Capparis spinosa* L. (mean ± SD)

Compound	% of inhibition ± SD
Chloroform extract	12.50 ± 5.18
Ethanol extract	58.60 ± 2.62
Water extract	17.00±4.07
Positive control Suramin	79.00%

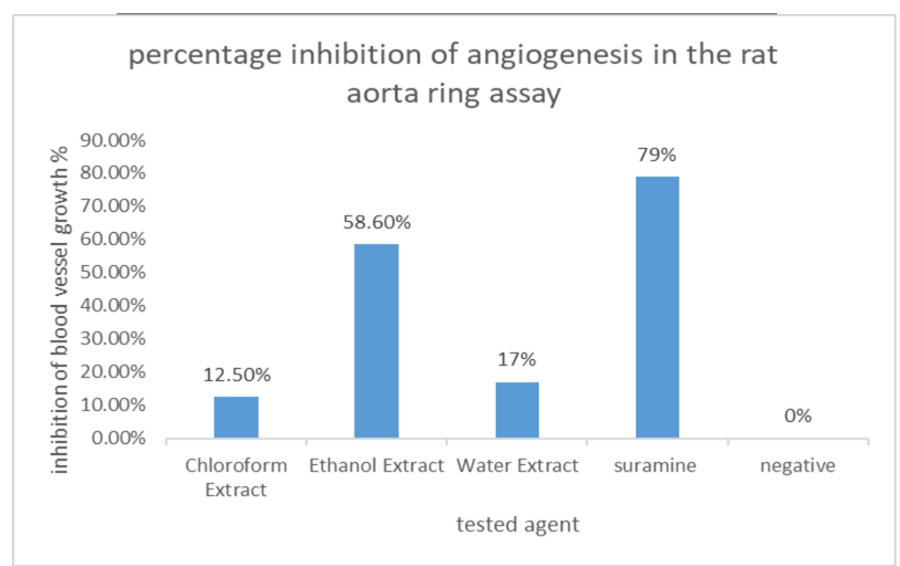

Figure 1: Anti – angiogenesis activity of 100µg/ml of each of chloroform, ethanol and water extracts along with the negative controls in ex vivo aortic ring model

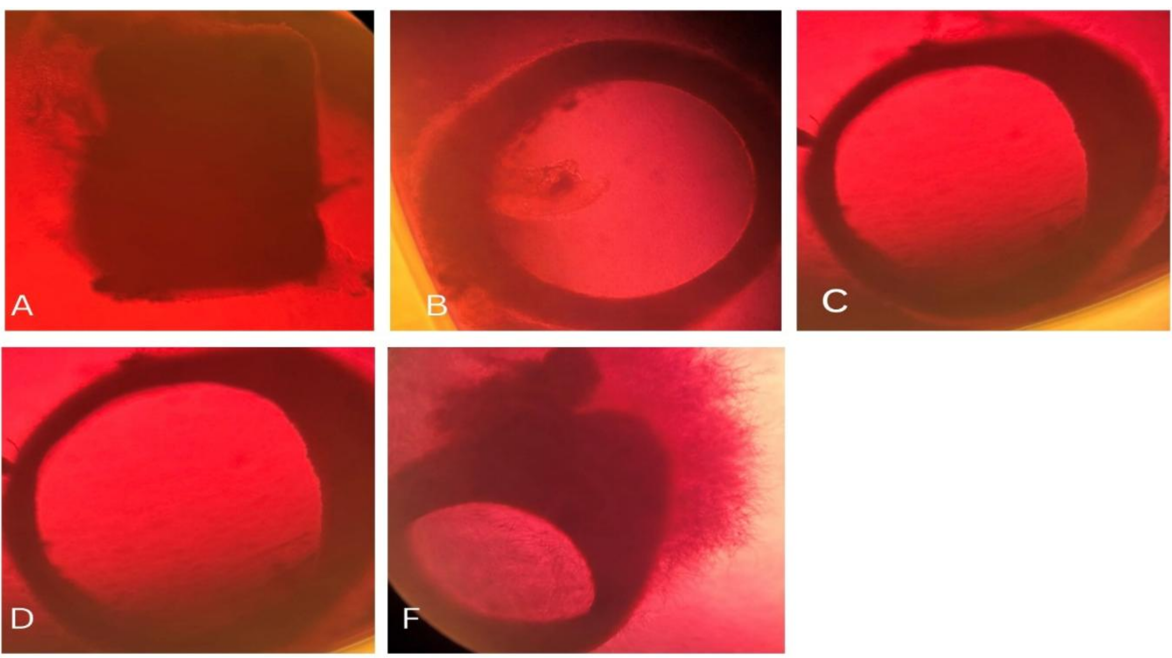

Figure 2: Representative microscopic images of rat aortic rings illustrating microvessel outgrowth following treatment with (A) chloroform extract, (B) aqueous extract (C) ethanolic extract of Capparis Spinosa L. (100 µg/mL), (D) suramin (100 µg/mL) as a positive control and (E) 0.1% DMSO (as a negative control) on day 5 of incubation.

Effect of ethanolic extract of *Capparis spinosa L.* on angiogenesis in the in-vivo chick chorioallantoic membrane assay:

The (CAM) model revealed a significant anti-angiogenic effect of the ethanolic leaf extract derived from *Capparis spinosa* L. The CAMs subjected to treatment displayed a significant regression of blood vessels; specifically, a marked disruption of the normal branching pattern and the formation of an avascular zone surrounding the implanted filter paper disc, in contrast to the control group.

Based on a semi-quantitative scoring system, the extract produced a significant inhibitory response, which was reflected in a high inhibition score (+++), indicate extensive suppression of neovascularization.

Table 3: Semi-quantitative assessment of angiogenesis inhibition in the CAM assay following treatment with *Capparis spinosa* L. extract

Eggs numbers	Inhibition zone (mm)	Scoring
1	14	+++
2	13	+++
3	11	+++
4	10	+++
Mean ± SD	12±1.83	+++

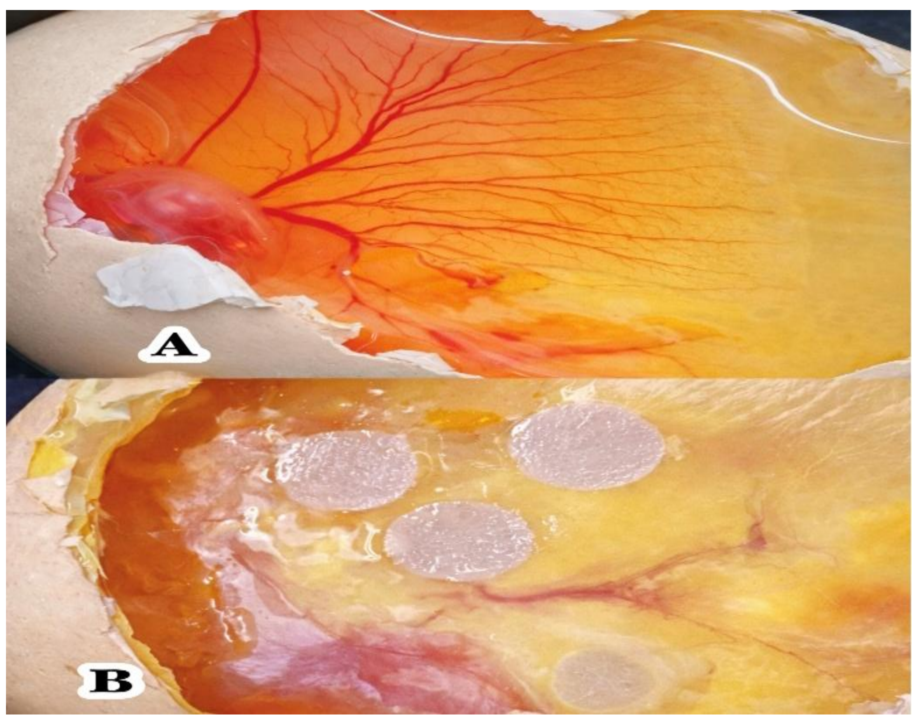

Figure 3: Effects of *Capparis Spinosa* L. on Neovascularization in Chorioallantoic Membrane of Chick Embryo (A: Control and B: CAM treated with *Capparis Spinosa* L. ethanolic leaf extract)

Antioxidant Activity

The antioxidant activity of the extract, as presented in Table 4, exhibited concentration-dependent DPPH radical scavenging effect. Increasing extract concentration was associated with enhanced radical scavenging capacity.

A gradual decrease in absorbance values was noted with increasing extract concentration, confirming enhanced radical scavenging activity at higher doses. These findings indicate that the antioxidant activity of the extract is dose-dependent.

The IC50 value was extrapolated to be approximately 123 µg/mL. However, this value exceeds the highest tested concentration, indicating moderate antioxidant activity.

Table 4: DPPH Radical Scavenging Activity of ethanolic extract of *Capparis spinosa* L.

Concentration (µg/mL)	Absorbance(Mean± SD)	DPPH scavenging %
100	1.724 ± 0.134	42.53
50	2.456 ± 0.034	18.14
25	2.694 ± 0.149	10.20
12.5	2.785 ± 0.110	7.17
6.25	2.798 ± 0.007	6.74
3.125	2.959 ± 0.023	1.36

DISCUSSION

Angiogenesis plays a critical role in both physiological and pathological conditions, particularly cancer, where excessive vessel formation supports tumour growth and metastasis [20]. Targeting angiogenesis has therefore become a key strategy in the development of therapeutic interventions [21]. Natural products, especially plant-derived bioactive compounds, have attracted attention due to their multi-target effects and relative safety compared to synthetic agents [22]. The ethanolic extract of *Capparis spinosa* demonstrated the strongest inhibitory effect on angiogenesis compared to chloroform and aqueous extracts, indicating that ethanol efficiently solubilizes bioactive flavonoids and phenolic compounds responsible for modulating endothelial function [23]. In the rat aortic ring (RAR) assay, the ethanolic extract significantly suppressed microvessel sprouting, suggesting a direct effect on endothelial proliferation and migration under controlled ex vivo conditions, consistent with previous findings reported by Sahib [24] demonstrating the anti-angiogenic activity of plant-derived extracts in similar models. Complementary evaluation using the chick chorioallantoic membrane (CAM) model confirmed these effects in a more complex in vivo environment, showing a marked reduction in blood vessel formation. The use of multiple discs on different regions of the CAM surface is consistent with previously reported CAM assay protocols, where localized (non-systemic) application allows evaluation of angiogenic response in separate vascular areas without interference [25]. This approach enhances spatial assessment of vascular inhibition while maintaining independent measurement for each application site. The consistent results across both models indicate that the extract's activity is robust and biologically relevant. These observations are supported by recent studies indicating that flavonoid-rich plant extracts inhibit angiogenesis through modulation of VEGF-mediated pathways, endothelial cell proliferation, and migration [26]. The flavonoids present in *C. spinosa*, including quercetin, kaempferol, and rutin, are reported to downregulate VEGF expression and suppress endothelial cell activation, which likely underlies the observed inhibitory effects [27]. The antioxidant properties of the ethanolic extract, as evidenced by the DPPH assay, may further contribute to angiogenesis inhibition. Oxidative stress is a known enhancer of VEGF signalling and endothelial activation, and reduction of reactive oxygen species can attenuate pro-angiogenic stimuli [28]. Previous research has shown similar results for plant extracts high in phenolic compounds, which had moderate antioxidant activity in DPPH and related tests [29].

This dual mechanism, direct interference with angiogenic signaling and mitigation of oxidative stress provides a plausible explanation for the robust inhibition observed in both RAR and CAM assays [30]. The use of complementary *ex vivo* and *in vivo* models strengthens the validity of the findings. The RAR assay offers precise measurement of microvessel sprouting in a controlled ex vivo system, while the CAM assay incorporates the complexity of an intact vasculature in vivo, capturing interactions that may not be fully represented in vitro [31]. The variation in screening concentration between the RAR and CAM assays is attributable to differences in tissue thickness, medium volume, and system complexity. RAR uses thin aortic rings and small culture volumes, allowing precise measurement of microvessel length, whereas CAM provides a more complex, thicker vascular bed where diffusion and local microenvironmental factors may alter effective concentration [32]. The present study represents part of a broader ongoing research project. The primary objective of this work was to evaluate the anti-angiogenic and antioxidant activities of the extract using established experimental models. Detailed qualitative identification of active constituents was beyond the scope of the current study and will be

addressed in subsequent investigations. Although these results are promising, the study has limitations. Notably, the specific bioactive compounds responsible for the anti-angiogenic activity were not isolated. Furthermore, direct molecular evidence of VEGF pathway modulation was not obtained. Future research should focus on isolating and characterizing the active constituents, as well as investigating their precise mechanisms of action using targeted molecular assays. Despite promising results, this study has some limitations. The sample size for certain assays was limited, which may affect statistical power. Findings are based on ex vivo and in vivo models and may not fully represent responses in human systems. Variability inherent to biological models, such as CAM and RAR assays, could influence quantitative results. Lastly, although the study demonstrates clear anti-angiogenic and antioxidant effects, the underlying molecular mechanisms were not fully elucidated and require further investigation. Future studies will focus on isolating specific bioactive compounds and evaluating their targeted effects on VEGF-mediated pathways and oxidative stress modulation.

CONCLUSION

The ethanolic extract of *Capparis spinosa* exhibits potent anti-angiogenic and antioxidant activities, demonstrating its potential as a natural therapeutic agent for disorders associated with pathological angiogenesis. Further mechanistic studies are required to fully understand the bioactive components and their molecular targets, providing a foundation for developing plant-based angiogenesis inhibitors.

The results showed that *Capparis spinosa* L. is a promising natural source of anti-angiogenic compounds that require further investigation.

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All authors contributed to the preparation of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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ETHICS STATEMENTS

Ethical approval was Obtained from ethics committee of Al-Nahrain University, College of Pharmacy (Approval No. SY/3/7/1012, dated in 23 November).

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النشاطان المضادان لتكوّن الأوعية الدموية والمضاد للأكسدة لنبات الكَبَر في نموذج حلقة الأبهر الجرذي ونموذج الغشاء الجنيني للبيضة

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الملخص
الخلفية: تكوّن الأوعية الدموية الجديدة من الأوعية الموجودة مسبقًا هو عملية بيولوجية أساسية ودقيقة التنظيم. تكوّن الأوعية ضروري لتجديد الأنسجة، التئام الجروح، ونمو الجنين في الظروف الفسيولوجية الطبيعية. ومع ذلك، فإن الاختلال في هذه العملية يساهم في تطور عدة أمراض، مما يجعلها هدفًا علاجيًا مهمًا. لذلك، حظيت المنتجات الطبيعية، بما في ذلك نبات الكبر، باهتمام كبير كعوامل مضادة لتكوّن الأوعية الدموية.

طرق البحث: هدفت هذه الدراسة إلى تقييم النشاط المضاد لتكوّن الأوعية والنشاط المضاد للأكسدة لمستخلصات أوراق نبات الكبر باستخدام نماذج حيوية مكملة. تم إجراء اختبار حلقة الأبهر للجرذ، واختبار الجذور الحرة، واختبار الغشاء الجنيني للبيضة لتأكيد النتائج في بيئة معقدة
النتائج: أظهر المستخلص الكحولي تثبيطًا ملحوظًا لنمو الأوعية الدقيقة في اختبار حلقة الأبهر، بقيمة تركيز نصف مثبط مقدارها 16.2 ميكروغرام/مل، كما قلل من تكوّن الأوعية الجديدة في نموذج الغشاء الجنيني للبيضة، مع منطقة تثبيط بمقدار 12 ± 1.83 ملم. كما أظهر المستخلص نشاطًا مضادًا للأكسدة يعتمد على التركيز في اختبار الجذور الحرة.
الاستنتاجات: أظهر المستخلص الكحولي لنبات الكبر أنشطة مضادة لتكوين الأوعية الدموية والمضادة للأكسدة ملحوظة، ويعد مصدرًا طبيعيًا واعدًا للمركبات القادرة على تعديل تكون الأوعية الدموية والإجهاد التأكسدي، مما يدعم إمكاناته التطبيقية العلاجية.
الكلمات المفتاحية: *Capparis spinosa*; النشاط المضاد للوعاء؛ النشاط المضاد للأكسدة؛ حلقة الأبهرية في الفئران؛ اختبار (CAM CAM) CAM