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Investigation of the Anti-Angiogenic activity of *Anabasis setifera*

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ABSTRACT

Objective : To find the potential anti-angiogenic and antioxidant effect of *Anabasis setifera* extracts.

Methods: . The rat aortic ring anti-angiogenesis assay, 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 aqueous extract of *Anabasis setifera* demonstrated significant inhibition of microvessel outgrowth in the rat aortic ring assay by 71% , with 86.5% reduction of blood vessels growth In the CAM model, treatment resulted in a marked reduction in neovascularization compared with the control group. The extract also exhibited concentration-dependent antioxidant activity in the (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay.

Conclusions: The water extract of *Anabasis setifera* . exhibits significant anti-angiogenic and antioxidant activities and may represent a promising natural source angiogenesis agents .

Keywords: Angiogenesis; *Anabasis setifera* ; Rat aortic ring; CAM assay; DPPH; Antioxidant activity.

INTRODUCTION

Generation of new blood vessels from an existing vascular network is a critical biological process required for embryonic development, tissue repair, and physiological regeneration⁽¹⁾. Under typical conditions in healthy adults, the vasculature is mostly quiescent, with endothelial cells having a very low mitotic frequency. This homeostatic state is maintained through a precise balance of pro- and anti-angiogenic factors⁽²⁾.

The endothelium is the deepest layer of all blood vessels, composed of a single layer of vascular endothelial cells (ECs). ECs are essential for maintaining a functional circulatory system by regulating vascular tone, permeability, coagulation, and inflammatory responses⁽³⁾. Recent advancements in single-cell genomics have revealed extraordinary variety and adaptability in ECs. These cells have organ-specific transcriptome fingerprints, indicating that they perform specialized tasks to meet the needs of the surrounding tissue⁽⁴⁾.

Angiogenesis is painstakingly regulated by a complex and dynamic combination of signalling molecules, cell-cell interactions, and environmental inputs. This regulation ensures that blood vessels expand just when and where they are required. The key concept of this regulation system is a balance of pro-angiogenic (growth-promoting) and anti-angiogenic (inhibitory) components. In healthy, quiescent tissues, inhibitory signals are predominant, ensuring vascular stability. Angiogenesis involves a significant shift in this balance, which is commonly referred to as the angiogenic switch^(5,6).

Anabasis setifera belongs to the family Amaranthaceae . greyish-green, scale-like leaves that are succulent and help conserve water and has gained increasing scientific interest due to its high content of bioactive secondary metabolites. Traditionally considered agricultural wast ⁽⁶⁾ , the *A.setifera* has emerged as a valuable source of pharmacologically active compounds with antioxidant, anti-inflammatory, antiproliferative, and vascular-modulating properties⁽⁷⁾

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The plant's remarkable adaptation to extreme environmental factors, such as drought, elevated temperatures, salinity, is primarily attributable to its expansive and deeply penetrating root system .

This ecological robustness is believed to affect its phytochemical composition; specifically, environmental stress frequently triggers the synthesis of protective secondary metabolites, encompassing phenolics and flavonoids^(8,9)

Although several medicinal plants have been investigated for anti-angiogenic activity, the anti-angiogenic potential of *Anabasis setifera* remains insufficiently explored . *A.setifera* was selected for the present study because it contains bioactive phytochemicals such as flavonoids, phenolics, Rutin , alkaloids, which have been associated with modulation of angiogenesis-related pathways. However, no previous study has specifically evaluated its effect on angiogenesis using CAM assay, DPPH. Therefore, this study aimed to investigate the anti-angiogenic activity of . *A.setifera* and address this gap in knowledge.

MATERIALS AND METHODS

Materials

In July 2025, *Anabasis setifera* plants were gathered from the Al-Najaf desert in Iraq. A reputable pharmacognosy specialist at the Al-Razi Center for Alternative Medicine confirmed the validity of the collected plant material to guarantee precise botanical identification and a dependable plant source.

Plant collection and authentication

Anabasis setifera were gathered, cleaned with running tap water to get rid of any sticking impurities, and allowed to dry for seven to ten days at room temperature before being processed into a fine powder using an electric grinder and sieved through a fine mesh screen to produce uniformly sized particles.

The powdered material was weighed using a digital precision balance and then extracted 150 g of *Anabasis setifera* and store it in an airtight plastic bag with silica gel to reduce the effects of moisture uptake and subsequent degradation, before extraction.

The extraction process was carried out in a Soxhlet apparatus, and the corresponding solvent was added to each of them at the following extraction conditions: distilled water at 100 °C, chloroform and 70% ethanol at approximately 60 °C. The extract was collected by filtering the mixture with Whatman No. 1 filter paper. Each extract was then concentrated using low pressure rotary evaporator at 40°C

to produce crude extracts and then kept for three days in the autoclave to confirm that the final extract is solvent-free . These crude extracts were stored in dry airtight containers to be used further in the experiment.

Anti-Angiogenic Evaluation of the *ex vivo* rat aortic ring Model.

The *ex vivo* rat aortic ring assay, as described by (Brown et al., 1996) and reorganized by (Blacher et al., 2003) (10), with slight methodological modifications to guarantee the accuracy and reproducibility of the experiment, was used to assess anti-angiogenic activity (Figure 1).

3 Adult albino male rats aged 12–14 weeks were humanely euthanized by inhalation of diethyl ether in accordance with institutional ethical and animal-care guidelines. The thoracic aorta was carefully excised under a dissecting microscope, immediately transferred into cold Hank's Balanced Salt Solution (HBSS), and gently cleaned of surrounding connective and adipose tissue. The aorta was then sectioned into uniform rings of approximately 1 mm thickness using a sterile scalpel. Approximately 20 rings were prepared from each thoracic aorta and distributed among the control and treatment groups under identical experimental conditions. Rings showing mechanical damage or luminal clot formation were excluded. Individual aortic rings were placed in 48-well tissue culture plates containing 300 µL of M199 medium supplemented with 20% heat-inactivated fetal bovine serum (HIFBS), ε-aminocaproic acid (0.1%), L-glutamine, gentamicin (0.6%). Fibrin clot formation was initiated by adding 10 µL of thrombin solution (50 NIH U/mL), followed by incubation at 37 °C in a humidified atmosphere with 5% CO₂ for 15 minutes. Subsequently, 300 µL of fibrinogen solution (3 mg/mL, prepared in serum-free M199) supplemented with aprotinin (5 µg/mL) was added to stabilize the fibrin matrix and support angiogenic sprouting.

Chloroform, ethanolic, and aqueous extracts of *Anabasis setifera* were prepared as stock solutions (10 mg/mL) in dimethyl sulfoxide (DMSO) under aseptic conditions. The stock solutions were subsequently diluted with M199 culture medium to obtain the required working concentration (100 µg/mL) for biological evaluation.

Tissue rings treated with 1% DMSO served as vehicle control. All treated groups were compared against the vehicle control (1% DMSO).

Cultures were incubated at 37 °C in a humidified atmosphere containing 5% CO₂ for five consecutive days. On day four, culture medium was replaced with great caution to maintain the nutrients and tissue viability throughout the angiogenic period.

Growth of the microvessels was observed on day five using an inverted microscope at 40× magnification and representative images were captured using a digital camera system and associated software. Experimental

operations were carried out under aseptic conditions. Plant extracts solutions were filtered aseptically using a 0.45 μm membrane filter followed by application of the solution with a syringe. To achieve methodological accuracy and reproducibility, prior determinations of experimental concentrations and experimental volumes were done. Angiogenic inhibition was expressed as mean percentage inhibition relative to the vehicle control \pm standard deviation (SD) and calculated using the following equation:

$$\text{Blood vessel inhibition (\%)} = \left[1 - \left(\frac{A_{\text{treated}}}{A_{\text{control}}} \right) \right] \times 100$$

Where:

A_control = mean of blood vessel growth in negative control rings (mm)

A_treated = mean of blood vessel growth in treated rings (mm)

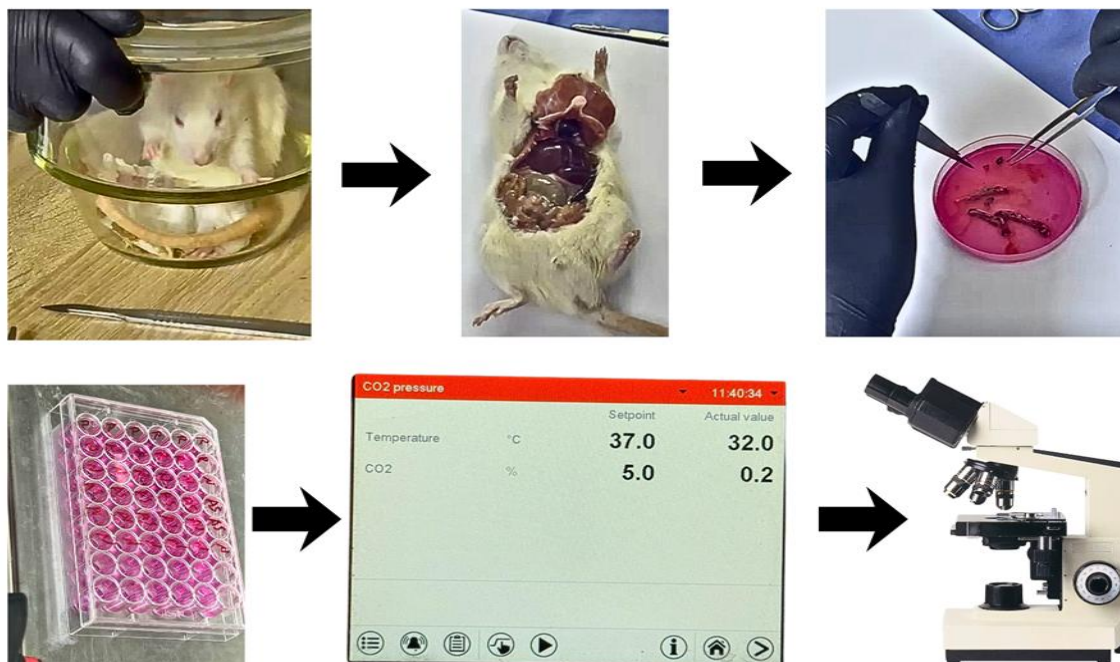


Figure 1: Anti-Angiogenic Evaluation of the ex vivo rat aortic ring Model

Chick Chorioallantoic Membrane (CAM) Assay

Fertilized chicken eggs used for CAM assay were purchased from local hatchery in Baghdad. eggs were surface disinfected with 70% ethanol to remove external contaminants and then placed horizontally in an incubator set at 37°C with 60% relative humidity under aseptic conditions to enhance the initial development of chorioallantoic membrane (CAM) as shown in Figure 2.

the first day of incubation was considered day 0.

On day 4 of incubation, 2 ml albumin were aspirated out through a pinpoint hole punctured down by the side and sealed to reduce internal pressure and facilitate detachment of the CAM from the shell, then eggs were incubated for further 24 hours. On day 7 of incubation, a circular window measuring approximately 3-4 cm in diameter was carefully created in the eggshell under aseptic conditions to expose the chorioallantoic membrane (CAM). To achieve the required working concentration, a stock solution (10 mg/mL) made in 1% dimethyl sulfoxide (DMSO) was diluted. After being impregnated with 50 μL of the extract solution, sterile filter paper discs (5 mm in diameter) were gently placed onto the CAM surface without causing any disruption to the surrounding vasculature and allowed to air dry. The eggs were put back in the incubator for a further 48 hours after the window was sealed. CAM photos were taken using an inverted microscope at low magnification on day 9 of incubation (48 hours after treatment) under uniform illumination. Two groups were used in the experiment: one received the aqueous extracts of *Anabasis setifera* while the other was given 1% DMSO as a negative control. There were six eggs in each group (n = 6) based on the statistical power of the study and in accordance with previously published CAM assay studies that used a similar experimental design.

The number of branching blood vessels radiating toward the filter disc inside a specified circular area centered on the disc was used to quantify angiogenesis. To guarantee uniformity across samples, the same region size was applied to every CAM image. To ensure uniform counting, digital grid overlays were placed after images were analyzed using ImageJ software (National Institutes of Health, USA). Mean \pm standard deviation (SD) was used to express the data. The following formula was used to determine the percentage blockage of angiogenesis:

Inhibition (%) is equal to $(1 - [\text{Mean vessel count of treated group} / \text{Mean vessel count of negative control}]) \times 100$.



Figure 2: Chick Chorioallantoic Membrane (CAM) Assay

(1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay

The antioxidant activity of aqueous extract of *Anabasis setifera* was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay, as described⁽¹¹⁾. A 0.1 mM DPPH solution was freshly prepared by dissolving 39.4 mg of DPPH in 1 L of methanol in an amber volumetric flask. The solution was gently mixed until complete dissolution and protected from light throughout the experiment.

A stock solution of *Anabasis setifera* extract was prepared by diluting 100 μL of the extract with 900 μL of methanol to obtain a final volume of 1 mL. Serial dilutions were then prepared from the stock solution to obtain concentrations of 500, 250, 125, 62.5, and 31.25 $\mu\text{g}/\text{mL}$.

The assay was carried out in 96-well microplates by adding 100 μL of each extract concentration to 200 μL of the DPPH solution. Methanol alone served as the blank, while methanol mixed with the DPPH solution served as the negative control. All samples were tested in triplicate. Plates were gently mixed and incubated at room temperature in the dark for 30 minutes.

Absorbance was measured at 517 nm using an ELISA microplate reader. The decrease in absorbance indicated the scavenging of DPPH radicals by antioxidant constituents in the extract. The percentage of DPPH radical scavenging activity was calculated according to the following equation⁽¹¹⁾

$$\text{Scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 is the absorbance of the control (DPPH solution without sample) and A_1 is the absorbance of the sample after reaction with DPPH.

RESULTS

Extraction process

Chloroform, ethanol, and distilled water were used in a Soxhlet apparatus to extract 150 grams of the dried powdered material of *Anabasis setifera*. Depending on the solvent used for the extraction method produced three crude extracts with varying yields. The following formula was used to get the extraction yield (%) based on the original dry weight of the plant material used (150 grams).

$$\text{Extraction yield (\%)} = (\text{weight of extract (gm)} / \text{weight of initial dry powder material}) \times 100$$
, as shown in Table 1.

Table 1: Weight and extraction yield of crude extracts obtained from *Anabasis setifera*

Type of extract	Weight of extract	Extraction Yield (%)
Chloroform	1.25 gm	0.833%
Ethanol	3.5 gm	2.33%
Water	20.3 gm	13.53%

The water showed the highest yield percentage at 13.53%, whereas the chloroform showed the lowest yield at 0.833%.

Rat aortic ring assay screening of the Anti-angiogenic Activity of *Anabasis setifera* Extracts on rat aorta ring assay

Anabasis setifera extracts' anti-angiogenic potential was assessed using the ex vivo rat aortic ring (RAR) assay. At a dosage of 100 µg/mL, aortic rings were treated with chloroform, ethanolic, and aqueous extracts. Suramin (100 µg/mL) served as the positive control, while 1% DMSO served as the negative control. Measurements of microvessel outgrowth were made on the fifth day of incubation as shown in Table 2. Water extract significantly inhibit blood vessels in comparison to NC $P < 0.05$, Water extract significantly inhibit blood vessels in comparison to chloroform / ethanol $P < 0.05$ and There were no significant difference between chloroform / ethanol extract $P > 0.05$. Data were analyzed using a two-tailed t-test.

The aqueous extract had the strongest anti-angiogenic activity (70.48%), followed by the chloroform extract (51.43%), the lowest inhibitory activity (47.62%) was observed with the ethanolic extract. The assay's validity was confirmed when suramin showed a noticeable inhibitory effect on angiogenesis, as anticipated (Figure 3).

Table 2: Screening of the anti-angiogenic activity of *Anabasis setifera* extracts using the rat aortic ring assay

Compound (Extract)	Concentration (µg/mL)	Blood vessel growth (Mean) ±sd	Inhibition (%)
Chloroform extract	100	5.1 ± 1.5	51.43%
Ethanol extract	100	5.5 ± 1.7	47.62%
Water extract	100	3.1 ± 1.4	70.48%
Negative control (NC)	0.01	10.5 ± 1.8	0
Positive control (suramin)	100	1.70±0.50	85.5%

NC: negative control

SD: standard deviation

* $p < 0.05$ vs NC

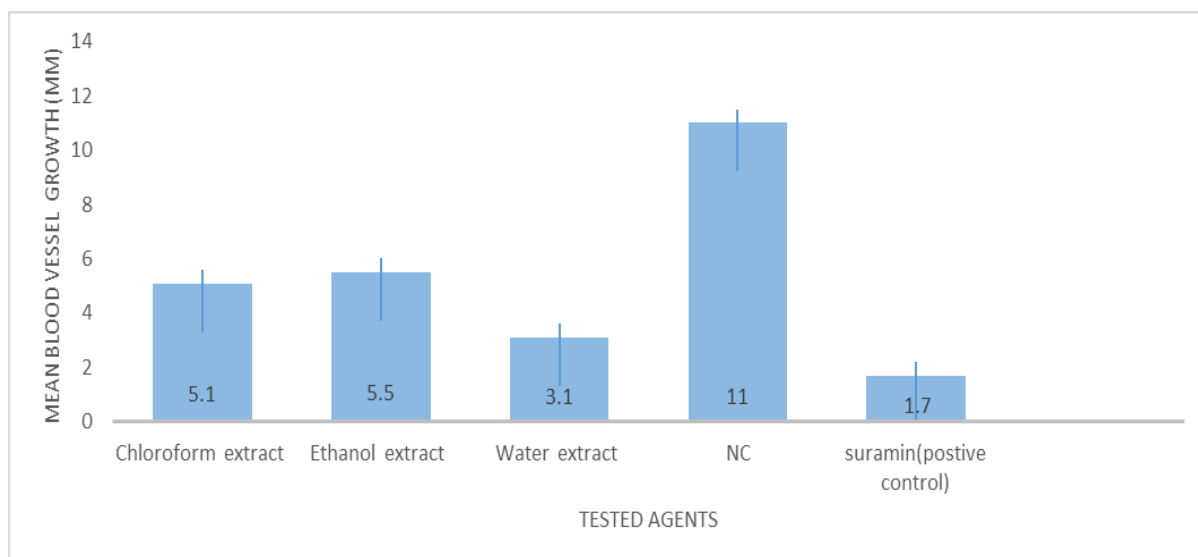
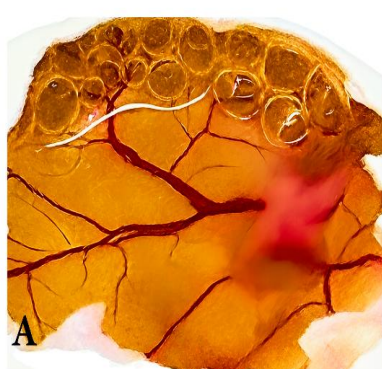


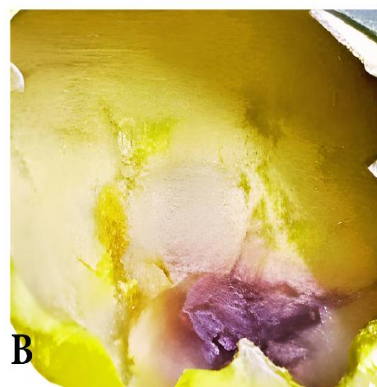
Figure 3: Effect of different *Anabasis setifera* extracts on blood vessel growth using the rat aortic ring assay. Chloroform, ethanol, and aqueous extracts significantly reduced microvessel outgrowth compared with the negative control (NC).

In vivo CAM Assay (Chick Chorioallantoic Membrane Assay)

By counting the visible blood vessels in the treated area and comparing the mean blood vessel count between the treated and negative control groups, the anti-angiogenic effect was evaluated in the CAM test. The findings demonstrated that *Anabasis setifera's* aqueous extract clearly inhibits the development of blood vessels. The treated group exhibited fewer blood vessel growth than the negative control group, as seen in Figure 4. While the treatment group clearly shown inhibition of neovascularization, the control group displayed normal and dense vascularization. This reduction corresponds to an angiogenesis inhibition of nearly 86.5%, indicating a strong suppressive effect of the extract on neovascularization. The quantitative analysis in Figure 3 supported this result. The mean blood vessel count decreased from 10.07 ± 1.617 in the negative control group to 1.367 ± 0.306 in the treated group (Figure 5). This decrease confirms the anti-angiogenic activity of the aqueous extract in the CAM model.



**Negative control
1% Dimethyl sulfoxide (DMSO)**



**After Treatment
With ANABASIS SETIFERA
Aqueous Extract**

Figure 4: Representative CAM images illustrating the reduction in vascular density (A) Negative control and (B) After treatment with *Anabasis setifera* aqueous extract.

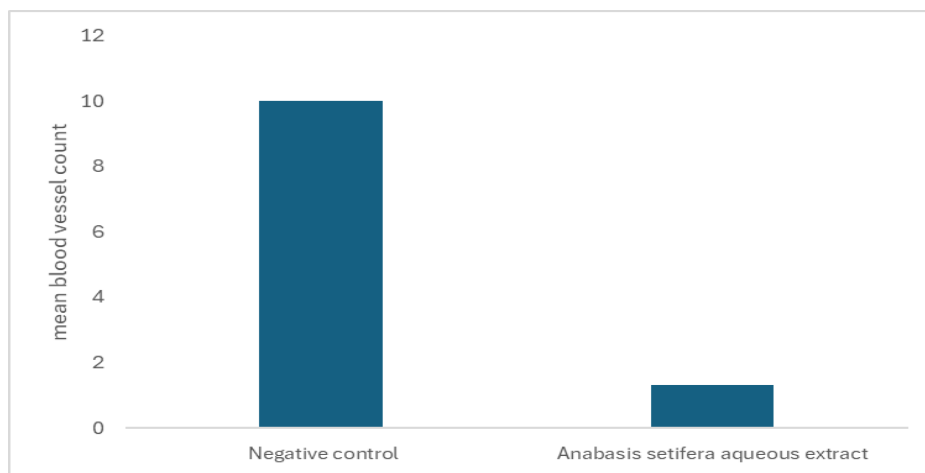


Figure 5: Quantitative comparison of the mean blood vessel count in the CAM assay.

Antioxidant Activity (DPPH Radical Scavenging Assay)

The aqueous extract of *Anabasis setifera* showed significant DPPH radical scavenging activity in a concentration-dependent manner ($P \leq 0.05$). As presented in Table 3, the highest scavenging activity was observed at 500 $\mu\text{g/mL}$ (91%), with a progressive decrease in antioxidant activity at lower concentrations. Non-linear regression analysis of the dose–response curve revealed an IC_{50} value of approximately 150.6 $\mu\text{g/mL}$. As shown in Figure 6, the percentage of DPPH radical scavenging activity was calculated using the absorbance of the control (DPPH solution without extract), which was measured experimentally using ELISA and found to be 2.9.

Table 3: DPPH Radical Scavenging Activity aqueous extract of *Anabasis setifera*

Concentration ($\mu\text{g/mL}$)	DPPH Scavenging (%)
500	91
250	63
125	41
62,5	18
31,25	9

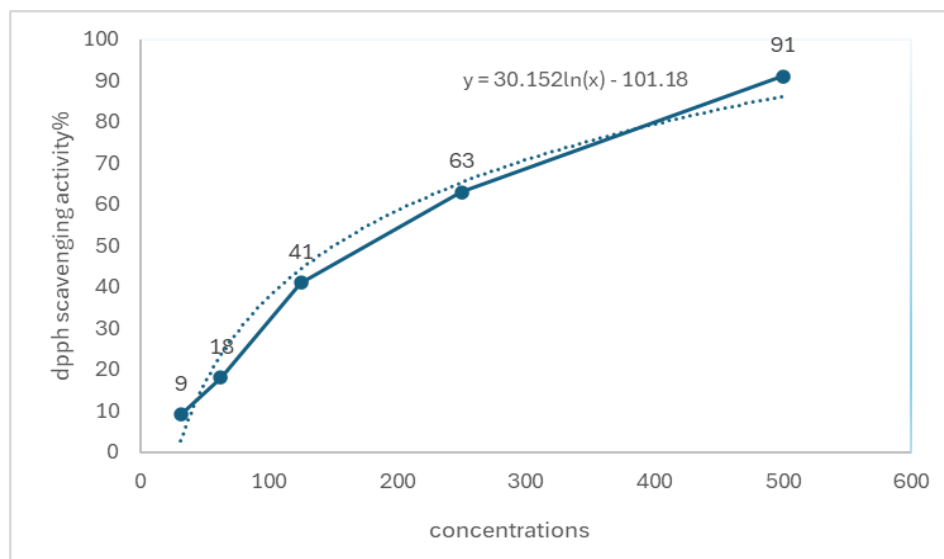


Figure 6: Dose–response curve of DPPH radical scavenging activity of the aqueous extract of *Anabasis setifera*

Discussion

Angiogenesis process consider target for scientist in inventing new agents as a method to treat a related diseases. The data from this study revealed that aqueous extract of *A. setifera* was significantly inhibitor for blood vessels. This data may be attributed to the bioactive constituents. Mohammadi showed *Anabasis setifera* contains phenolic compounds, flavonoids (rutin) and triterpene saponins with antioxidant and anti-inflammatory activities, which may contribute to anti-angiogenic potential⁽¹²⁾. These phytochemicals are widely reported to suppress angiogenesis through modulation of vascular endothelial growth factor (VEGF) signaling pathways. reduce oxidative stress and reactive oxygen species (ROS), which are major stimulators of VEGF expression in tumor and inflammatory cells. In addition, these compounds may suppress hypoxia-inducible factor-1 alpha (HIF-1 α), a key transcription factor responsible for VEGF production under hypoxic conditions, Therefore, the biological activity of *A. setifera* may contribute to anti-angiogenic effects via inhibition of VEGF expression and endothelial cell proliferation⁽¹³⁾.

in this study authors decided to conduct a series of investigation including (RAR) assay, cam and dpph

du to the novelty of this herb . dpph has been done to confirm the anti oxidant activity and cam assay was mandatory to be conducted because its considered as *in vivo* model for angiogenesis evaluation and demonstrated significant inhibition of blood vessel growth.

Extraction Yield

Water had the highest extraction yield among the investigated solvents, followed by ethanol and chloroform . This finding implies that polar phytochemical compounds are abundant in the plant, whereas non-polar components are found in comparatively smaller quantities⁽¹⁴⁾ Consequently, it seems that the type of chemicals extracted from the plant and the extraction efficiency are significantly influenced by the polarity of the solvent.

The anti – angiogenic activity in ex vivo rat aorta ring assay and dose response study for *Anabasis setifera*

In the present study, the ex vivo rat aortic ring (RAR) assay was employed to evaluate the anti-angiogenic potential of *Anabasis setifera* crude extracts. This assay can be deemed as a good experimental model to investigate angiogenesis since it is an experimental model that recapitulates the complex process of microvessel sprouting in a controlled environment also due to its reproducibility, cost-effectiveness, simplicity, and strong correlation with *in vivo* assays⁽¹⁵⁾

. A concentration of 100 $\mu\text{g}/\text{mL}$ was used to test the chloroform extract, ethanol extract and aqueous extract with suramin being used as the positive control and 1% DMSO used as the negative control.

The findings indicated that all the tested extracts have a significant inhibitory effect on microvessel outgrowth compared to the vehicle control ($p < 0.05$), which proves that *Anabasis setifera* contains anti-angiogenic components. The inhibitory effects were different in the extracts and this indicates that the nature of solvent is a critical factor that is used in extracting compounds that inhibit angiogenesis.

The aqueous extract has the greatest anti-angiogenic potential, This activity could be due to the presence of polar bioactive compounds e.g. phenolics, flavonoids and glycosides which are known to disrupt angiogenesis-related pathways e.g. endothelial cell proliferation and migration⁽¹⁶⁾.

The chloroform extract had medium inhibitory activity which means that the non-polar components could be terpenoids or lipophilic secondary metabolites contributing to the preventive angiogenic effect of the plant to a lesser degree than the polar compounds.

Conversely, the lowest inhibitory activity was observed with the ethanolic extract indicates that the semi-polar fraction might have fewer active constituents in the inhibition of angiogenesis or a combination of compounds with lower biological activity⁽¹⁷⁾.

The negative control (1% DMSO) did not inhibit (0%), the maximum growth of microvessels was observed which proves that the positive effects were provided by the plant extracts but not the solvent. Moreover, the expected result of suramin, namely a sharp inhibitory effect testified to the experimental model and justified the assay reliability.

Generally, these results indicate that *Anabasis setifera*, and especially its aqueous extract, have good anti-angiogenic potential. It is suggested to conduct further research in order to isolate and identify the active phytochemicals and study their mechanisms of action .

Anabasis setifera aqueous extract In vivo chick chorioallantoic membrane (CAM) assay.

The CAM assay is the most widely used in vivo angiogenesis assay .It offers a sound vascular structure in which to study tumor growth, angiogenic and anti-angiogenic molecules. Other advantages of CAM assay are, simplicity and cost-effectiveness; also because the CAM assay is a closed system, the half-life of many experimental molecules, such as small peptides, tends to be much longer compared to animal models, allowing the experimental study of potential anti-metastatic compounds⁽¹⁸⁾

The outcome of the CAM assay indicated that the aqueous extract of *Anabasis setifera* contains a distinct anti-angiogenic effect. The treated group had lower blood vessel formation compared to negative control group, which indicates that the angiogenesis is inhibited. This was evident in the CAM images whereby the treated group had less vascularization.

This finding was also proved by the quantitative analysis. The average number of blood vessels fell by the negative control group of 10.07 ± 1.617 to the treated group of 1.367 ± 0.306 . Such sharp decrease indicates that the aqueous extract was effective in inhibiting new blood vessels growth.

Overall, the results of the current study suggest the aqueous extract of *Anabasis setifera* is an important anti-angiogenic agent in the CAM model. The mechanism of action and active compounds should be identified in future studies in order to explain the observed anti-angiogenic activity, and the active compounds may be bioactive secondary metabolites including flavonoids, phenolic compounds and polyphenols, which are frequently reported in medicinal plants. These compounds have been evidenced to inhibit angiogenesis in a variety of ways, such as the down regulation of vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and matrix metalloproteinases (MMPs), which are all known to be major pro-angiogenic agents in endothelial cell growth, migration, and extracellular matrix remodelling⁽¹⁹⁾.

Free Radical Scavenging Activity (DPPH Radical Scavenging Assay)

The DPPH assay is a widely used method for evaluating antioxidant activity by measuring the ability of compounds to scavenge free radicals. Free radicals are highly reactive and unstable molecules that are implicated in the development of various degenerative diseases, including mutagenesis, carcinogenesis, cardiovascular disorders, and aging-related processes⁽²⁰⁾.

The results of this investigation showed that aqueous extract of *Anabasis setifera* had strong DPPH radical scavenging activity that was concentration-dependent. From 9% at 31.25 $\mu\text{g/mL}$ to 91% at 500 $\mu\text{g/mL}$, the percentage inhibition gradually rose, demonstrating a distinct dose–response association. This pattern is typical of antioxidants produced from plants and shows that the concentration of the extract has a significant impact on its ability to neutralize free radicals.

The logarithmic regression model applied to the dose–response data yielded an IC_{50} value of 150.6 $\mu\text{g/mL}$. The IC_{50} parameter is widely regarded as a reliable quantitative indicator of antioxidant potency, reflecting the efficiency of a compound or extract in reducing 50% of DPPH radicals⁽¹⁹⁾. The obtained IC_{50} value (150.6 $\mu\text{g/mL}$) indicates a moderate antioxidant activity according to previously published DPPH reference values. In comparison, standard antioxidants such as ascorbic acid commonly exhibit much lower IC_{50} values, ranging from approximately 3–38 $\mu\text{g/mL}$, reflecting their stronger free radical scavenging activity. The relatively higher IC_{50} observed for the plant extract may be attributed to its complex phytochemical composition and the synergistic interactions among its constituents⁽²¹⁾.

The presence of phenolic compounds, flavonoids, tannins, and other secondary metabolites with electron-transfer and hydrogen-donating properties is responsible for the reported antioxidant action.

Since oxidative stress is linked to the development of many chronic illnesses, such as cancer, heart disease, and neurological problems, the extract's antioxidant potential in this study emphasizes its potential for therapeutic use.⁽²²⁾

The reduction in blood vessel formation observed in the treated groups may be explained by the high antioxidant activity of *A. setifera*. The plant is rich in phenolic compounds and flavonoids, which are known to scavenge reactive oxygen species (ROS). Excess ROS normally stimulates angiogenesis by activating hypoxia-inducible factor-1 alpha (HIF-1 α), leading to increased expression of vascular endothelial growth factor (VEGF). By reducing oxidative stress, the phytochemical constituents of *Anabasis setifera* may suppress HIF-1 α stabilization and subsequently decrease VEGF production⁽²³⁾.

Overall, the results confirm that the aqueous extract of *Anabasis setifera* has significant antioxidant activity in a concentration-dependent manner.

CONCLUSION

The aqueous extract of *A. setifera* demonstrated potent inhibition of blood vessel formation in both ex vivo rat aortic ring and in vivo CAM models. The extract significantly reduced neovascularization and microvessel outgrowth compared to the control groups. The DPPH experiment showed that the extract was a good free radical scavenger, which means it has antioxidant potential. The extract's antioxidant properties may be partly responsible for the anti-angiogenic activity that was noted. The results show that *Anabasis setifera* is a promising natural source of anti-angiogenic compounds that needs to be studied more.

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The authors declare that all authors contributed equally to the writing and preparation of this manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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ETHICS STATEMENTS

Obtained from ethical committee of Al-Nahrain University, College of pharmacy letter No SY/3/7/1012 ,dated in 23 November 2025

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التحري عن النشاط المضاد لتشكّل الأوعية الدموية لنبات *Anabasis setifera*

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الخلاصة

الهدف: إيجاد التأثير المحتمل المضاد لتشكّل الأوعية الدموية والمضاد للأكسدة لمستخلصات نبات عُجْرَم الشوك

الطرائق: أُجريت مقارنة حلقة الشريان الأبهر للجرذ للوقوف على التثبيط لعملية تشكّل الأوعية الدموية، ومقايضة اقتناص الجذور الحرة (دي بي بي إتش)، ومقايضة الغشاء المشيمي السقائي لجنين الدجاج (كام)، وذلك في مختبر زراعة الأنسجة التابع لقسم الأدوية في كلية الصيدلة بجامعة النهريين

النتائج: أظهر المستخلص المائي لنبات عُجْرَم الشوك تثبيطاً كبيراً لنمو الأوعية الدموية الدقيقة في مقارنة حلقة الشريان الأبهر للجرذ بنسبة 71 بالمئة، مع انخفاض بنسبة 86.5 بالمئة في نمو الأوعية الدموية في نموذج الغشاء المشيمي السقائي لجنين الدجاج، حيث أسفر العلاج عن انخفاض ملحوظ في تشكّل الأوعية الدموية الجديدة مقارنة بالمجموعة الضابطة. كما أظهر المستخلص نشاطاً مضاداً للأكسدة يعتمد على التركيز في مقارنة اقتناص الجذور الحرة

الاستنتاج: يمتلك المستخلص المائي لنبات عُجْرَم الشوك أنشطة بارزة مضادة لتشكّل الأوعية الدموية ومضادة للأكسدة، وقد يمثل مصدراً طبيعياً واعداً للعوامل العلاجية المحاربة لتشكّل الأوعية الدموية

الكلمات المفتاحية: تشكّل الأوعية الدموية، عُجْرَم الشوك، حلقة الشريان الأبهر للجرذ، مقارنة الغشاء المشيمي السقائي، اختبار اقتناص الجذور الحرة، النشاط المضاد للأكسدة.