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Evaluation of Anti-angiogenic Activity of Iraqi *Mesembryanthemum cordifolium* using rat aorta ring assay

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Abstract

New blood vessels are produced during angiogenesis, which is necessary in both normal and abnormal physiological and pathologic states. The use of non-toxic plant extracts that inhibit angiogenesis might lead to more focus on the use of natural sources as a pivotal therapeutic agent in the treatment of many diseases and malignancies. *Mesembryanthemum cordifolium* is belongs to the Aizoaceae family and commonly known as the Heartleaf Ice Plant or Tiny Sun Rose. The plant was cultivated in the city of al Diwaniyah. No previous research has been conducted on this plant and its antiangiogenic properties. Hence, this research aimed to assess the antiangiogenic capacity of the Iraqi *M. cordifolium*. For this study, male albino rats aged 12 to 14 weeks were used. With a Soxhlet apparatus, the defatted plant components were extracted in 85 % methanol for 12 h. Subsequently, each fraction was fractionated using petroleum ether, chloroform and ethyl acetate and each fraction underwent phytochemical analysis. Rat aortic rings were infused with 100 µg/mL of each plant component and placed in a growth medium. In addition, the dose-response relationship was assessed for the most active fraction at 6 different concentrations ranging from 6.25 µg/mL to 100 µg/mL. Compared to the negative control (1 % DMSO), the results demonstrated that fractions of *M. cordifolium* inhibit the growth of blood vessels in a dose-dependent manner; chloroform demonstrates the most potent effect. The results indicate that the chloroform fraction derived from *M. cordifolium* has a good ability to limit blood vessel development. This makes it a viable option for future investigation as an angiogenesis inhibitor.

Keywords

angiogenesis; chloroform; *Mesembryanthemum cordifolium* ; phytochemical analysis

Introduction

Mesembryanthemum cordifolium is belongs to the Aizoaceae family. Occasionally referred to as the heartleaf ice plant or little sun rose (1). This plant is indigenous to South Africa and is extensively grown as an ornamental species worldwide. The polysulfonated naphthylurea chemical suramin was first created as a trypanocidal medication. Its antitumor efficacy was shown recently (2). It has a lot of promise for cancer treatment because of its ability to disrupt the autocrine mechanisms that cause the formation of many human cancers. The chick chorioallantoic membrane's angiogenesis may be inhibited by suramin. Suramin might neutralize growth factor effects. It has

been shown that suramin prevents the interaction between growth factors and their receptors, including PDGF (Platelet-derived growth factor), basic FGF (Fibroblast growth factors) and vasculotropin. The suppression of VEGF function may mediate Suramin's anti-angiogenic action (3).

First things initially: let's go into the inner workings of this angiogenesis phenomenon. Imagine if a portion of your body is not receiving sufficient oxygen, this would be analogous to a plant that needs water (4). If this occurs, the body alarm system will activate. Doing so sets off a chain reaction, activating genes that need an increase in the number of blood vessels. The most powerful factor in this process is something known as HIFs, which stands for hypoxia-inducible factors. In the same way that supervisors on a building job issue instructions to establish more blood supply routes, they will do the same thing. A protein known as vascular endothelial growth factor-A (VEGF-A) is the primary agent in action here. It plays a role in developing and directing these new blood routes (5).

However, angiogenesis may not always have positive implications. Indeed, more than 70 illnesses are associated with it, particularly in instances such as ocular inflammation and cancer (6). Excessive accumulation of blood vessels may lead to a congested state, which is undesirable (7). The major participants in this process - a combination of growth factors, proteins and other substances- were identified by scientists almost four decades ago. VEGF, PDGF and a few additional factors are now receiving significant attention (8). These individuals are often responsible for causing this congestion. In the overall scheme of cancer, regulating angiogenesis might be likened to cutting the supply routes to the tumor. Cancer cells proliferate and disseminate via co-opting blood arteries. If we can find methods to inhibit or slow this process of vessel formation, it may have the ability to save lives by starving the cancer cells of nutrients. This is the reason for the increased interest in anti-angiogenic therapies. Not only do they directly attack the cancer cells, but they also destroy their vital connections. It resembles a dual-pronged attack strategy, generating optimism in our ability to minimize the effects of cancer (9).

Materials and Methods

Plant material

The Mesembryanthemum cordifolium was collected in June 2023 from al-diwanyah City, in south-central Iraq, as shown in (Fig. 1).

The taxonomic identification of the plant was done by Assist. Prof. Dr. Israa Abdul Razzaq Majeed, Department of Biology and College of Science, University of Baghads. The plant was dried at ambient temperature for 16 days, (10, 11) crushed using an electric blender, weighed and then subjected to extraction (12). A 100 g quantity of the dehydrated plant has been crushed into a fine powder. The powder was then macerated using n-hexane for 24 h to remove fat and unwanted material from the plant (13); after that, it was extracted with 85 % methanol using a Soxhlet apparatus (14) and to get a clear extract, it was filtered using the filter paper from Whatman No. 1. To concentrate the extract, a vacuum-assisted rotary evaporator (15) was used. Subsequently, the crude extract was diluted with the minimum quantity of distilled water and then subjected to sequential partitioning using the liquid-liquid fractionation method depending on polarity. This was accomplished using a separatory funnel, petroleum ether (PE) with (a boiling point of 60-80 °C), chloroform (C) and ethyl acetate (EA) as partitioning solvents. Each fraction was obtained using 250 mL of solvent. The procedure was repeated multiple times. Following that, the 3 fractions were subjected to drying with anhydrous sodium sulfate, followed by filtration and evaporation to remove moisture using a rotary evaporator. The resulting residue was then weighed and designated for further analysis. The phytochemical analysis conducted on the crude extract and its respective fractions revealed distinctive constituents in each fraction. The analysis indicated the presence of steroids in the petroleum ether fraction, alkaloids in the chloroform fraction and both flavonoids and phenolic compounds in the ethyl acetate fraction.

Fig. 1. Iraqi *Mesembryanthemum cordifolium*.

Animal

The experimental design and dealing with animals were conducted following the requirements outlined in the "Research Ethical Approval Form" and as per the protocol that was authorized by Baghdad University/College of Pharmacy, Baghdad, Iraq with the ethical approval number RECAUBCP1092023G on 10/9/2023. Male albino rats aged 12 to 14 weeks were used.

Rat aorta ring anti-angiogenic ex vivo assay (RAR)

The experiment was carried out at the tissue culture laboratory. The angiogenesis test used in this method is similar to the one developed earlier, but with minor alterations (16).

Preparation of rat aorta rings from albino rats

Aortic ring tissue cultures from rats were created using the standard techniques reported (17). Male Sprague Dawley rats were used to make aortic rings. The aortas were cut into cross pieces of 1 mm in length and were meticulously washed many times using Hanks balanced salt solution (18). The experiment was conducted using 48-well tissue culture plates manufactured by Costar Corning in the United States. Each well was supplemented with 500 µL of serum-free M199 growth medium with 3 mg/mL of fibrinogen, an ingredient that helps promote the development of blood clots to avoid hemorrhaging. Additionally, 5 µg/mL of aprotinin was used to prevent the breakdown of blood clots. Each tissue slice was positioned at the midpoint of the well, together with 10 mL of thrombin (50 NIH U/mL) in 0.15 M sodium chloride was introduced to achieve a gel-like environment around the aorta portion. Following the placement of the piece of the blood artery among the fibrin gels, 0.5 mL of medium M 199, which included 20 % heat-inactivated fetal bovine serum (HIFBS), 0.1 % aminocaproic acid, 1 % L-Glutamine and 60 µg/mL gentamicin (to prevent contamination), was introduced into each well. Three distinct plant extract solutions were produced using DMSO as the solvent. The extracts were introduced into the wells to check their influence on the development of blood vessels. Each extract was tested 6 times to ensure consistency. Stock solutions with a 10 mg/mL concentration were prepared for the 3 fractions (petroleum ether, chloroform and ethyl acetate). Each sample was dissolved in dimethyl sulfoxide (DMSO) and subsequently dilution in M199 medium to achieve the desired last concentration of 1 %. The media was supplemented with plant extracts at 100 µg/mL. This addition was repeated 6 times. The seeded rings were cultured at 37 °C and 5 % CO_2 using a humidified incubator to replicate the conditions inside a rat's body. After 4 days, the medium on top was changed to a newly produced medium, as described earlier. Certain wells were assigned as negative controls, consisting of the solvent without plant extracts. Furthermore, Suramin, a well-established anti-angiogenic substance, was Utilized as a reference standard for comparison purposes.

On the 5th day, the results were analyzed under a microscope. Blood vessel proliferation was quantified using a digital imaging device and computer at a magnification of 10X. The quantification of blood vessel inhibition was assessed using the methodology established by Nicosia and his team (19). The findings are shown as the mean % of inhibition relative to the negative control. The process was replicated twice, with 6 duplicates of each sample. The inhibition % was determined using the prescribed formula (20).

$$\text{Blood vessels inhibition \%} = 1\text{- (A0/A)} \times 100$$

Here, A0 is the mm measured distance of blood vessel expansion in the sample material, and A is the mm measured distance of blood vessel development in the negative control.

Dose-response analysis using the rat aorta ring test on the most effective fraction

After identifying the most effective fraction of *M. cordifolium*, a concentrated solution was created using DMSO. Subsequently, a series of dilutions were made in M199 medium (including 1 % DMSO) at 200, 100, 50, 25, 12.5 and 6.25 µg/mL concentrations. The wells designated as negative controls were exposed to a medium containing 1 % DMSO.

Statistical Analysis

The results were reported as mean ± SD (standard deviation). An analysis based on statistics was conducted using a one-way ANOVA followed by a Tukey post-hoc test (t–test) and considered significant at $P < 0.05$. The IC_{50}, which represents the concentration of a substance required to inhibit blood vessel growth, cell multiplication, and tube formation by 50 %, was determined using a logarithmic equation. This equation was derived from a graph plotting fraction concentration in µg/mL against the inhibition %. In the equation, Y represents the inhibition % and X represents the concentration. The statistical analysis was conducted using SPSS version 21.0.

Results and Discussion

The aortic rings, subjected to 100 µg/mL of the 3 extracts, placed in the entire growth medium of M199, exhibited a notable variance in blood vessel inhibition, as seen in Table 1.

Table 1. Fractions blood vessel growth inhibition percentage

Samples	% of inhibition (Mean ± SD)
Petroleum ether fraction	76 ± 0.6
Chloroform fraction	91 ± 0.4
Ethyl acetate fraction	55 ± 1.3
Negative control (DMSO 1 %)	0
Positive control (suramin)	100

The tests showed that the petroleum ether, chloroform and ethyl acetate fractions significantly slowed down the growth of blood vessels on the 5th day of the tests, compared to the negative control ($p<0.05$). Out of these fractions, the chloroform fraction exhibited the most potent antiangiogenic activity, with a 91 % reduction in blood vessel formation. The petroleum ether fraction followed

with a 76 % inhibition, while the ethyl acetate fraction showed a 55 % inhibition. Furthermore, there was an equivalent effect seen for both the chloroform % and the standard (suramin), as shown in Fig. 1-3.

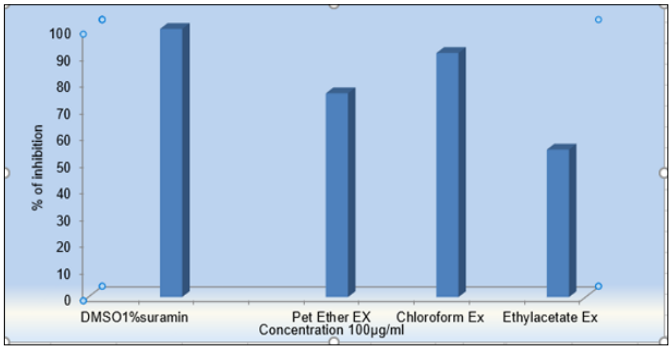

Fig. 2. Fractions' anti-angiogenesis activity at a concentration of 100 µg/mL.

The dose-response curve was established by administering 6 different concentrations of chloroform (the most potent portion) to the implanted rat aortic rings. The fraction exhibited a substantial and dose-dependent inhibition of angiogenesis compared to the negative control ($p<0.001$) on the 5th day Table 2 and Fig. 3 respectively).

Based on the logarithmic equation (y= 0.9351X - 2.0222), as seen in Fig. 4, the IC_{50} was determined to be 55.63 µg/mL. X represents the concentration and Y is the inhibition %.

According to this research, the chloroform fraction of *M. cordifolium* had the most antiangiogenic effects. The fact that these fractions, particularly chloroform, inhibited blood vessel formation at a rate of 91 % highlights the promise of chemicals derived from plants in the treatment

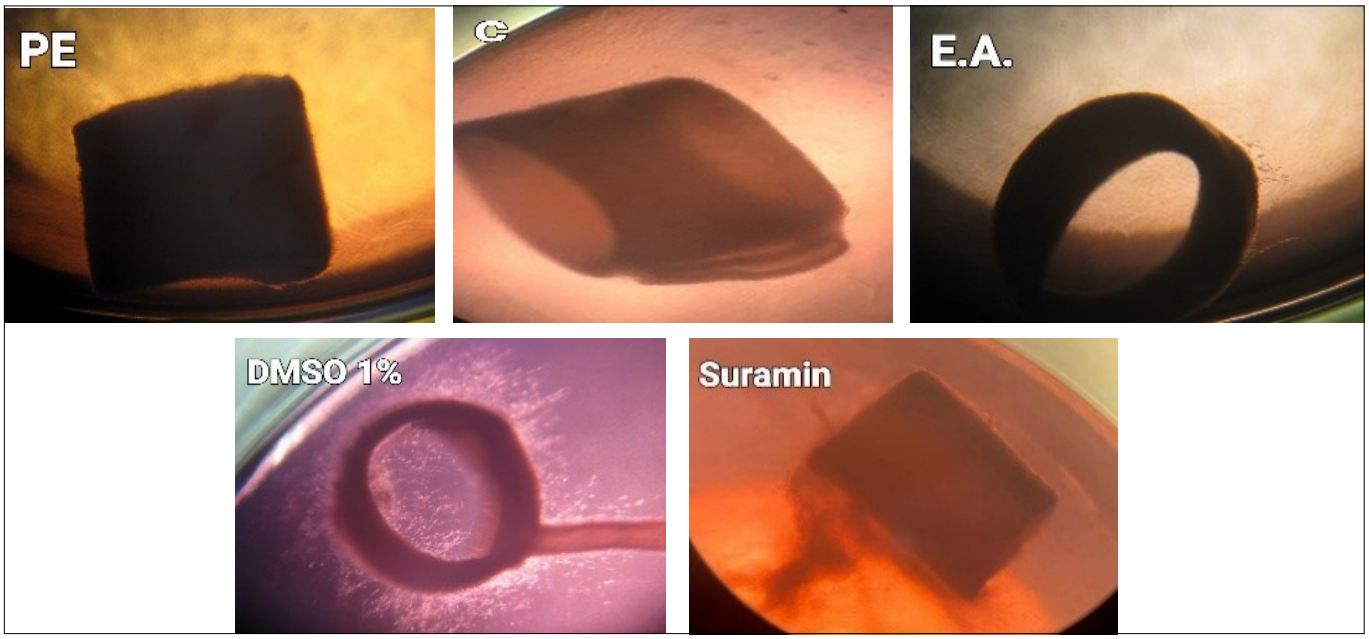

Fig. 3. The anti-angiogenesis action of 100 µg/mL of *Mesembryanthemum cordifolium* fractions, **P.E**.: petroleum ether, **C**: chloroform, **E.A**.: ethyl acetate, **DMSO1 %** (negative control) and **suramin** (standard) in Aortic ring model *ex vivo*.

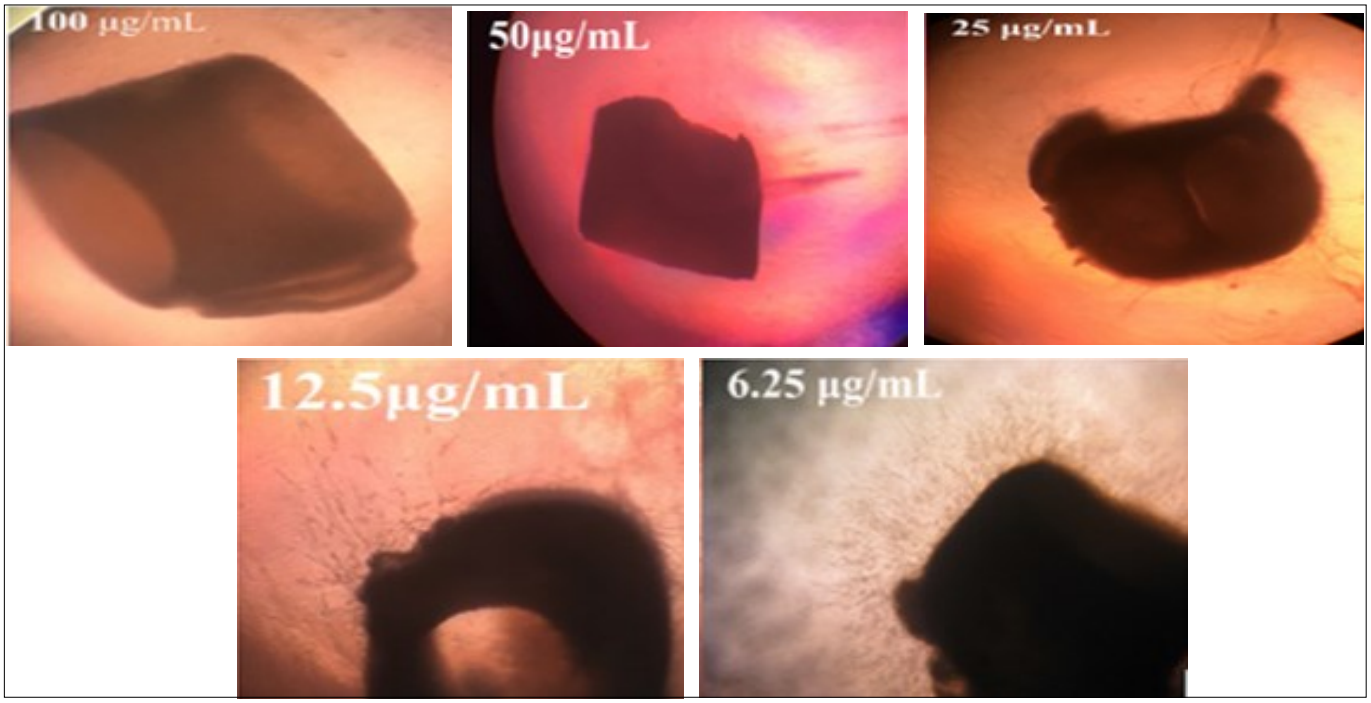

Fig. 4. Anti-angiogenesis effect of chloroform fraction in different concentrations (serial dilution).

Table 2. % of inhibition (Mean ± SD) of the serial dilution of chloroform fraction

Concentration (µg/mL)	% of inhibition (Mean ± SD)
200	91 ± 0.4
100	91 ± 0.4
50	78 ± 0.4
25	23 ± 0.3
12.5	18 ± 0.6
6.25	8 ± 0.8

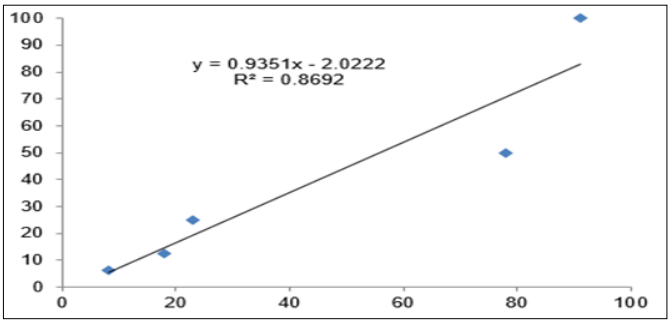

Fig. 5. Dose-response curve of chloroform fraction.

of diseases associated with angiogenesis. Previous research has shown that plant extracts may effectively modulate angiogenic pathways (21) and the chloroform fraction's dose-dependent suppression of angiogenesis (IC_{50} value of 55.63 µg/mL) correlates with this. Furthermore, it is a noteworthy discovery that *M. cordifolium* contains alkaloids, as confirmed by general alkaloidal testing and this investigation. This matches along with the increasing amount of data that suggests alkaloids produced from plants might be useful in cancer treatment. Furthermore, the results are consistent with literature publications, which indicate that alkaloids play a critical role in antiangiogenic action (22). Alkaloids have many biological functions, including their role as powerful angiogenesis inhibitors, which may explain why they have anticancer effects (23). It is also useful to compare the antiangiogenic activity of suramin, (24) (the positive control), with that of extracts from *M. cordifolium.* The chloroform fraction had the same impact as suramin, which means that substances produced from plants might be a safer, more natural substitute for synthetic antiangiogenic medicines (25). A potential new approach to cancer therapy is the use of Chinese medicinal herbal extracts, which have strong anti-angiogenic properties. These extracts work by inhibiting the formation of new blood vessels. The bioactive components included in these extracts, including alkaloids, flavonoids and saponins, can target various pathways. One of these pathways is the downregulation of angiogenic factors such as VEFG. As a result, these extracts show great promise as multifunctional therapeutic agents for disorders that rely on angiogenesis. (26) By modulating metabolic and signaling pathways, the secondary metabolite from plant extract influences angiogenesis, microtubule assembly creation inhibition, and cell death (27). Alkaloids can treat cancer due to their antiproliferative and cytotoxic actions on different types of cancer cells, as mentioned in literature reviews (28). Literature surveys indicate that *M. cordifolium* contains much higher concentrations of alkaloids. Alkaloids are among the chemicals with the greatest potential for biological action (29). However, researchers have isolated specific alkaloidal components from the genus *Mesembryanthemum*, and reports suggest that this plant tested positive for alkaloids in general (30). A large number of alkaloids have antiproliferative and cytotoxic effects on cancer cell lines derived from different histological sources (31). According to this research, alkaloids derived from natural sources show great promise as compounds that have great potential for treating various illnesses.

Conclusion

Natural compounds have the potential to combat cancer by preventing angiogenic problems and enhancing the effects of other anticancer treatments, such as chemotherapy and radiation. Compounds found in *Mesembryanthemum cordifolium* could potentially help treat diseases like cancer, where too many blood vessels can make the condition worse. However, we still need more studies to understand exactly how these compounds work to stop the growth of new blood vessels and ensure they're safe and effective for treating people. Early findings suggest that *M. cordifolium* holds great potential as a medicine, but further research is necessary to ensure its proper application for enhancing people's health.

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Compliance with ethical standards

Conflict of interest: No conflict of interest has been disclosed by the author.

Ethical issues: The experimental design and dealing with animals were conducted following the requirements outlined in the "Research Ethical Approval Form" and as per the protocol that was authorized by Baghdad University/ College of Pharmacy, Baghdad, Iraq with the ethical approval number RECAUBCP1092023G on 10/9/2023. male albino rats aged 12 to 14 weeks were used.

References

1. Lee JH, Nam SY. Comparison of growth and leaf color quality of *Mesembryanthemum cordifolium* f. *variegata* as affected by shading levels. Journal of People, Plants and Environment. 2023 Jun 1;26(3):207-17. https://doi.org/10.11628/ksppe.2023.26.3.207
2. Waltenberger J, Mayr U, Frank H, Hombach V. Suramin is a potent inhibitor of vascular endothelial growth factor. A contribution to the molecular basis of its antiangiogenic action. J Mol Cell Cardiol. 1996;28(7):1523-29. https://doi.org/10.1006/jmcc.1996.0142
3. Kreimeyer A, Müller G, Kassack M, Nickel P, Gagliardi ART. Suramin analogues with a 2-phenylbenzimidazole moiety as par-

tial structure; potential anti HIV- and angiostatic drugs, 2: Sulfanilic acid, benzenedisulfonic acid and naphthalene trisulfonic acid analogues. Arch Pharm (Weinheim) [Internet]. 1998 Mar 1 [cited 2024 Feb 15];331(3):97-103. Available from: https://europepmc.org/article/MED/9557135

4. Qassim RH, Kadhem EJ. Phytochemical investigation and anti-angiogenic examination of Iraqi *Vigna radiata* L. seeds and sprouts. Iraqi Journal of Pharmaceutical Sciences. 2020 Dec 27;29(2):37-47. https://doi.org/10.31351/vol29iss2pp37-47

5. Carmeliet P. Angiogenesis in life, disease and medicine. Nature [Internet]. 2005 Dec 15 [cited 2024 Feb 15];438(7070):932-36. Available from: https://pubmed.ncbi.nlm.nih.gov/16355210/

6. Khamees A, Khadim E, Sahib H. Investigation of the possible anti-angiogenic activity of Iraqi *Scabiosa palaestina* L. using *ex vivo* rat aorta ring assay. Journal of Complementary Medicine Research. 2021;12(4):249. https://doi.org/10.5455/jcmr.2021.12.04.37

7. Haseeb Khamees A, Jawad Kadheem E, Bahaa Sahib H, Hussein Ahmed O. A review on medicinal plants with antiangiogenic activity available in Iraq. J Pharm Res Int. 2019 Nov 30;1-10. https://doi.org/10.9734/jpri/2019/v31i630331

8. Khamees AH, Abdulhussein AJ, Sahib HB, Fawzi HA. Anti-angiogenic and antioxidant activity of Iraqi *Cyperus rotundus* ethanol extract. International Journal of Pharmacology. 2018;14(4):546-52. https://doi.org/10.3923/ijp.2018.546.552

9. Petrovic P. Targeting angiogenesis in cancer treatments: Where do we stand ? Journal of Pharmacy and Pharmaceutical Sciences [Internet]. 2016 Jun 26 [cited 2024 Feb 16];19(2):226-38. Available from: https://pubmed.ncbi.nlm.nih.gov/27518172/

10. View of A pharmacognostic approach, including phytochemical and GC-MS analysis, targeted towards the authentication of *Strobilanthes jomyi* P. Biju, Josekutty, Rekha & J.R.I.Wood [Internet]. [cited 2024 Mar 4]. Available from: https://horizonepublishing.com/journals/index.php/PST/article/view/2104/1916

11. View of phytochemical screening and gas chromatography-mass spectrometry analysis on *Ischaemum pilosum* Kleinex Willd. [Internet]. [cited 2024 Mar 4]. Available from: https://horizonepublishing.com/journals/index.php/PST/article/view/2349/2422

12. View of investigation of phytochemical constituents, GC-MS, DPPH free radical scavenging assay and mineral contents of *Glochidion sphaerogynum* (Mull. Arg.) Kurz bark extract [Internet]. [cited 2024 Mar 4]. Available from: https://horizonepublishing.com/journals/index.php/PST/article/view/2019/1879

13. Khamees AH, Kadhim EJ. Isolation, characterization and quantification of a pentacyclic triterpinoid compound ursolic acid in *Scabiosa palaestina* L. distributed in the North of Iraq. Plant Science Today [Internet]. 2022 Jan 1 [cited 2024 Feb 14];9(1):178-82. Available from: https://horizonepublishing.com/journals/index.php/PST/article/view/1398

14. Brahma S, Mochahary B, Kalita M, Goyal AK. Pharmacognostic and physicochemical characterisation of potential plants for antidiabetic herbal formulations. Plant Science Today [Internet]. 2022 Aug 3 [cited 2024 Mar 4];9(sp2):1-7. Available from: https://horizonepublishing.com/journals/index.php/PST/article/view/1704

15. View of phytochemicals analysis and antioxidant potential of hydroalcoholic extracts of fresh fruits of *Pistacia atlantica* and *Pistacia khinjuk* [Internet]. [cited 2024 Mar 4]. Available from: https://horizonepublishing.com/journals/index.php/PST/article/view/2853/2776

16. Brown KJ, Maynes SF, Bezos A, Maguire DJ, Ford MD, Parish CR. A novel *in vitro* assay for human angiogenesis. Lab Invest [Internet]. 1996 Oct 1 [cited 2024 Feb 16];75(4):539-55. Available from: https://europepmc.org/article/MED/8874385

17. Zhu WH, Guo X, Villaschi S, Nicosia RF. Regulation of vascular growth and regression by matrix metalloproteinases in the rat aorta model of angiogenesis. Lab Invest [Internet]. 2000 [cited 2024 Feb 16];80(4):545-55. Available from: https://pubmed.ncbi.nlm.nih.gov/10780671/

18. Sahib HB. The anti-angiogenic and anti-proliferative activity of methyl hydroxychalcone. Asian Pacific Journal of Cancer Prevention. 2022;23(6):2071-77. https://doi.org/10.31557/APJCP.2022.23.6.2071

19. Nicosia RF, Lin YJ, Hazelton D, Qian XH. Endogenous regulation of angiogenesis in the rat aorta model. Role of vascular endothelial growth factor. Am J Pathol [Internet]. 1997 Nov 1 [cited 2024 Feb 16];151(5):1379-86. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/pmid/9358764/?tool=EBI

20. Ali ZK, Sahib HB. Antiangiogenic activity of sweet almond (*Prunus dulcis*) oil alone and in combination with aspirin in both *in vivo* and *in vitro* assays. Asian Pacific Journal of Cancer Prevention. 2022 Apr 1;23(4):1405-13. https://doi.org/10.31557/APJCP.2022.23.4.1405

21. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. The Nucleus [Internet]. 2022 Dec 1 [cited 2024 Mar 2];65(3):399. Available from: /pmc/articles/PMC9579558/

22. Alasvand M, Assadollahi V, Ambra R, Hedayati E, Kooti W, Peluso I. Antiangiogenic effect of alkaloids. Oxid Med Cell Longev. 2019;2019. https://doi.org/10.1155/2019/9475908

23. Thawabteh A, Juma S, Bader M, Karaman D, Scrano L, Bufo SA *et al*. The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. Toxins (Basel). 2019 Nov 11;11(11). https://doi.org/10.3390/toxins11110656

24. Meyers MO, Gagliardi AR, Flattmann GJ, Su JL, Wang YZ, Woltering EA. Suramin analogs inhibit human angiogenesis *in vitro*. Journal of Surgical Research. 2000 Jun 15;91(2):130-34. https://doi.org/10.1006/jsre.2000.5920

25. Cragg GM, Newman DJ. Plants as a source of anti-cancer agents. J Ethnopharmacol. 2005 Aug 22;100(1–2):72-79. https://doi.org/10.1016/j.jep.2005.05.011

26. Wang S, Zheng Z, Weng Y, Yu Y, Zhang D, Fan W *et al*. Angiogenesis and anti-angiogenesis activity of Chinese medicinal herbal extracts. Life Sci. 2004 Apr 2;74(20):2467-78. https://doi.org/10.1016/j.lfs.2003.03.005

27. Sharifi-Rad J, Dey A, Koirala N, Shaheen S, El Omari N, Salehi B *et al*. *Cinnamomum* species: Bridging phytochemistry knowledge, pharmacological properties and toxicological safety for health benefits. Front Pharmacol [Internet]. 2021 May 11 [cited 2024 Mar 3];12:600139. https://doi.org/10.3389/fphar.2021.600139

28. Mondal A, Gandhi A, Fimognari C, Atanasov AG, Bishayee A. Alkaloids for cancer prevention and therapy: Current progress and future perspectives. Eur J Pharmacol. 2019 Sep 5;858:172472. https://doi.org/10.1016/j.ejphar.2019.172472

29. Said A, Attia E, Abdelmohsen U, A Fouad M. Natural products potential of the genus *Aptenia*. Journal of Advanced Biomedical and Pharmaceutical Sciences. 2019 Mar 5;0(0):0-0. https://doi.org/10.21608/jabps.2019.6908.1032

30. Waweru W, Wambugu FK, Mbabazi R. Evaluation of anti-inflammatory activity of *Aptenia cordifolia* leaves extract in wistar albino rats. J Pharmacogn Phytochem. 2017;

31. Tang J, Feng Y, Tsao S, Wang N, Curtain R, Wang Y. Berberine and coptidis rhizoma as novel antineoplastic agents: A review of traditional use and biomedical investigations. J Ethnopharmacol [Internet]. 2009 Oct 29 [cited 2024 Feb 16];126(1):5-17. Available from: https://pubmed.ncbi.nlm.nih.gov/19686830/