RESEARCH PAPER

Antiangiogenic Potential of Four Medicinal Plant Extracts in Comparative Evaluation with Chick Embryo Chorioallantoic Membrane (Cam) Assay

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Received: 5th Apr, 2026 | **Accepted:** 24th Apr, 2026 | **Available Online:** 26th Apr, 2026

ABSTRACT

The formation of new blood vessels or angiogenesis is important to tumor growth and metastasis. Cancer treatment seems to be promising when the process is halted. It is evident in the literature that the traditional medicinal plants possess established anti-angiogenic and anticancer effects. The present investigation involved testing and comparing the anti-angiogenic property of four herbal extracts, namely, Aloe barbadensis Miller (Aloe vera), Ocimum sanctum (tulsi), Zingiber officinale (ginger), and Curcuma longa (turmeric) on the chick chorioallantoic membrane (CAM) assay. The CAM of the fertilized chicken eggs was treated with ethanol extracts of the selected plant parts at two doses: 50 mg/kg and 100 mg/kg. Vascular branching patterns, vessel area, total vessel length, branch points, lacunarity, and suppression of neovascularisation were used to quantify angiogenic activity. All the plant extracts showed anti-angiogenic activity in a dose-dependent manner. The strongest activity was observed with Curcuma longa, which generated significant decreases in the vessel area, vessel length, and branching with a corresponding increase in the lacunarity (score = 3.6 -0.20100 mg -1, p = 0.001). The same pattern was shown in Zingiber officinale. The least effect was Aloe vera and Ocimum sanctum exhibited significant effects on angiogenesis inhibition. To verify the ocular observations and CAM scores, the image underwent quantitative analysis. Zingiber officinale and Curcuma longa are potent natural inhibitors of angiogenesis that may be beneficial as supplemental treatments for angiogenesis-related disorders. It is advised that more molecular research be done to clarify the underlying causes.

Keywords: The angiogenesis process, Herbal extracts, Ocimum sanctum, Curcuma longa, Zingiber officinale, Aloe vera, Chick CAM assay, and cancer treatment.

How to cite this article: Seal R, Habiba U, Mandal D, Karmakar A, Ghosh I, Aktar S, Ghosh I, Halder P. Antiangiogenic Potential of Four Medicinal Plant Extracts in Comparative Evaluation with Chick Embryo Chorioallantoic Membrane (Cam) Assay. Int J Drug Deliv Technol. 2026;16(31s):1118-1126. DOI: 10.25258/ijddt.16.31s.123

Source of support: Nil.

Conflict of interest: The authors declare no conflict of interest.

Introduction

Folkman researched tumor angiogenesis to understand its workings, and Hertig first used the term "angiogenesis" in 1935. The body uses angiogenesis, or the formation of new capillaries from preexisting vessels, as a vital physiological mechanism for both reproduction and repair.

A large amount of research has been done on the molecular and cellular processes that promote blood vessel creation, as well as the genetic foundations. Vasculogenesis and angiogenesis work together to create the vasculature throughout embryonic development (Lugano et al. 2020). It is a strictly regulated process that, aside from wound healing,

embryonic development, and corpus luteum formation, hardly ever takes place under typical circumstances. Tumor start and progression are significantly influenced by a number of variables that control the angiogenesis process. Angiogenic factors that promote the development of new blood vessels include bFGF, HGF, VEGF, hyaluronidase, collagenase, and MMP (Galvão et al. 2013). Nowadays, it is widely acknowledged that angiogenesis is a prerequisite for tumor growth and that each increase in tumor size necessitates an increase in vascular development. Without angiogenesis, tumors stay dormant indefinitely, and once they have a blood supply, they develop rapidly logarithmically. The tumor angiogenic switch seems to be induced when the balance between the angiogenic inhibitors and stimulators is shifted towards the pro-angiogenic environment (Liu et al., 2025). The hypothesis of the angiogenic switch, which relies on the rise in the synthesis of one or more positive angiogenic regulators, grew out of the assumption that the process of angiogenesis is regulated by the balance between molecules that have positive and negative regulatory influences (Ribatti et al., 2003). One of the most important prerequisites for carcinogenesis is still tumor angiogenesis, which is the process by which new blood vessels are formed through a complex system involving several endothelial cell-stimulating and inhibitory molecules (Lorenc et al., 2024). Cancer cells may not receive enough oxygen and nutrients as they proliferate and travel farther away from the blood arteries that supply them (Oktavia et al. 2017). In order to start angiogenesis, cancer cells release tumor angiogenesis factors (TAFs) in certain conditions (Saman et al. 2020). One TAF that promotes angiogenesis is basic fibroblast growth factor (bFGF) (Zahra et al. 2021). Once the cancer cells begin communicating with TAFs to enhance angiogenesis, it is easy to see this process in action when an excessive amount of bFGF is present in the tissue. The chorioallantoic membrane (CAM) model of the chick embryo is commonly used in vivo to determine angiogenesis and its suppression (Oktavia et al. 2017). It consists of two developmental structures which include the chorion and allantois. Developing chick egg chorioallantoic membranes are also commonly used in multiple medical fields and research areas to study angiogenesis, development, tumours, and the effects of therapeutic drugs. It is an angiogenic reaction to implant, which occurs 72-96 hours after stimulation as the vessels radially converge toward the center like spokes on a wheel (Marshall et al. 2020). On the other hand, the vessels surrounding the implant become less thick or even vanish when an angiostatic substance is tried. Drug screening from plant extract samples can be done by quantifying vessels in many CAM models.

Nowadays, a number of herbal remedies are used in cancer patients' treatment, such as podophyllotoxin derivatives, taxol analogues, and vinca alkaloids (vincristine, vinblastine) (Chaudhary et al. 2024). Several in vitro and in vivo studies have examined plants' anti-cancer and anti-angiogenic qualities (Iranmanesh et al. 2018). In industrialized cultures, herbal plants have important roles in the healthcare system (Sofowora et al. 2013).

Tulsi (Ocimum sanctum), a member of the Lamiaceae family of plants, is frequently referred to as "the elixir of life" and is believed to prolong life. The fresh leaves and stems of O. sanctum contain several important phenolic compounds, such as apigenin, isothymusin, cirsilineol, circimaritin, rosameric acid, and a trace quantity of eugenol. Among the volatile oils included in O are carvacrol, eugenol, methyl eugenol, and the sesquiterpene hydrocarbon caryophyllene. sanctum leaves. According to Arya et al. (2024), leaves also contain a variety of flavonoids, such as luteolin, orientin, and vicenin. Numerous pharmacological activities have been reported for Tulsi, including anticancer, antioxidant, anti-inflammatory, anti-stress, free radical scavenger, anti-diabetic, antileishmanicidal, central nervous system (CNS) depressant, anticoagulant, ulcer protective, antifungal, hepatoprotective, antihypertensive, cardioprotective, antiasthmatic, immunomodulatory, antifertility, antiulcer, antiviral, and antimicrobial activity. Most phytochemicals with an anti-angiogenic action include luteolin, apigenin, and rosmarinic acid (Pattanayak et al. 2010).

The perennial, drought-resistant succulent herb known as aloe vera (Aloe barbadensis Miller) is a member of the Asphodelaceae (Liliaceae) family. It is extensively spread in most regions of the globe such as Mexico, India, South America, Central America, Caribbean Islands, Australia, Africa, and Pacific rim countries and is considered an invasive species. Most prominent phytochemical constituents of A. vera include a variety of anthraquinones such as aloin, emodin, barbaloin, isobarbaloin, β-barbaloin and aloesone, many fatty acids, such as cholesterol, campesterol, β-sitosterol, and lupeol, and various enzymes, including aliiase, alkaline phosphatase, amylase, bradykinase, carboxypeptidase, catalase, cellulase, lipase, and peroxidase, have been found. In addition to these, it

contains hexuronic acid, mucopolysaccharides, glucosamines, galactouronic acid, chrysophanic acid, chrysamminic acid, aloetic acid, and homonataloin (Kaur and Bains 2024; Bhoopathy et al. 2024). The outer pericyclic tubules, known as aloe sap or aloe juice, and the interior parenchymatous tissue, known as aloe gel, contain the majority of the pharmacologically active components of aloe. Aloe vera's bioactive chemicals are very successful in treating burns, allergic reactions, rheumatoid arthritis, rheumatic fever, acid reflux, ulcers, diabetes, skin issues, dysentery, diarrhea, piles, and inflammatory digestive disorders. Strong anti-angiogenesis properties are demonstrated by aloe-emodin (Sánchez et al. 2020).

The Zingiberaceae family includes turmeric (Curcuma longa), which is widely grown in Asia's tropical regions. Numerous phytochemicals and minerals, including curcumin, demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols, are found in its root, which is its most widely used medicinal part (Fuloria et al. 2022). One of the best treatments for a number of illnesses, such as diabetes and skin disorders, is turmeric. Turmeric's effectiveness is attributed to its abundance of healthful phytoconstituents. One such phytoconstituent is curcumin, a nutraceutical compound with a wide range of pharmacological properties that have been demonstrated in both clinical and experimental settings. Its anti-inflammatory, cardioprotective, anti-mutagenic, anti-allergic, antioxidant, anti-hyperglycemic, and anti-angiogenic actions have been proven to be advantageous (Sharifi-Rad et al. 2020). The popular herbal spice ginger (Zingiber officinale Roscoe) is said to have come from either Southeast Asia or India. Because it is a sterile plant, it reproduces by rhizomes rather than seeds and thrives in tropical and subtropical climates worldwide. The primary phytoconstituents are various phenolic compounds, such as quercetin, zingerone, gingerenone-A, and 6-dehydrogingerdione, as well as gingerols, shogaols, and paradols. In addition, there are a number of terpene components that are thought to be the primary components of ginger essential oils, including β-bisabolene, α-curcumene, zingiberene, α-farnesene, and β-sesquiphellandrene. In addition, ginger contains lipids, organic acids, polysaccharides, and raw fibers (Mao et al. 2019). It is utilized in cooking, as a condiment or spice, and as a medicinal substance. Antipyretic, antiemetic, antioxidant, antiulcer, analgesic, hypotensive, antidiabetic, anti-inflammatory, and spasmolytic properties are well-established. According to reports, 6-gingerol and 6-shogaol exhibit anti-angiogenic qualities among all phytoconstituents. Therefore, the goal of the current investigation was to use four different herbal extracts to find the ideal dose for preventing angiogenesis during the growing of chicken embryos (Sharma et al. 2023).

Materials and Methods

Collection of Samples

This experiment included four distinct plant species. Two of these were the leaves of Tulsi and Aloe vera, while the other two were the fresh rhizomes of Ginger and Turmeric. The plant components were procured from the Bengal Institute of Pharmaceutical Sciences, Kalyani Ho, Kalyani, Nadia. The specimens were verified by the Botanical Survey of India, Acharya Jagadish Chandra Bose Indian Botanic Garden, Howrah, West Bengal.

Plant Extraction and Phytochemical Screening

The Tulsi, Ginger, and Turmeric plants were washed in running tap water for 2, 3 times and once with sterile water, air-dried, and then ground into granules. Next, 50 g of each powdered sample was soaked in 200 mL of ethanol for 48 hours at room temperature with occasional stirring. The mixture obtained was filtered through Whatman No. 42 filter paper, and the pure and homogeneous extract was dried into a powder form (Rawat et al. 2025). The gel was taken out from the leaves and then air-dried. For the Aloe vera leaf, the gel was extracted from the leaves and then air, dried. The dried product was macerated with 100 mL of sterile distilled water in a Waring blender for 10 minutes. The resulting mixture was first passed through a double layer of muslin cloth for coarse filtration, followed by centrifugation at 4,000 g for 30 minutes. The obtained supernatant was then filtered using Whatman No. 1 filter paper and subsequently heat sterilized (Chaudhary and Janmeda 2023). Successively extraction of 50 g of each of the air-dried and coarsely powdered plant materials with 200 mL of ethanol was made using a Soxhlet apparatus for 48 h. A Mantox heater supplied the heat required for solvent recycling. The filtrate of the obtained extract was made through a Whatman filter of Type 42 and then concentrated up to viscous consistency at 50 °C with the help of a rotary evaporator. The percentage yield was calculated by dividing the weight of the raw material by the weight of the extract, and then qualitative phytochemical analysis was performed as

described by Chaudhary et al. (2022). (Chaudhary et al. 2022).

Chorioallantoic Membrane (CAM) Assay

Fertilised white leghorn chicken eggs were obtained from the West Bengal Livestock Development Corporation Limited (WBLDC Ltd.). The CAM assay was performed with approval from the Institutional Animal Ethical Committee (No. IISER K/09/IAEC/2023, 24), Kolkata, India. The anti-cancer study has been carried out in accordance with the internationally accepted norms for animal research and is based on the 3Rs principle. It is also in compliance with the OECD TG 19 guideline.

The eggs were sanitized with 70% ethanol to remove contaminants and impurities. The broad end was oriented upwards during incubation at 37 °C and 60% humidity. The eggs were cycled thrice daily. After 2 or 3 days of incubation, the eggs were inspected with a handmade lamp, and the position of the embryo's head was recorded. 0.51 ml of albumin was extracted from the eggs with an 18-gauge hypodermic needle through a small aperture drilled at the narrow end of the eggs, facilitating the separation of the little CAM and yolk sac from the shell membrane. The portion of the shell encompassing the embryo air sac was excised using forceps, and the shell membrane beneath the air sac was eliminated. Subsequently, 50 mL of each herbal extract or sterile phosphate-buffered saline was given into the eggs of the plant for the treatment and sham control groups, respectively. The eggs were incubated again for 24 and 48 hours following the initial injection. Fifty microliters of the extracts were administered to the shell membrane. The study comprised five groups: group 1 (n=10), a phosphate-buffered saline infected control group; groups 2 (n=10), 3 (n=10), 4 (n=10), and 5 (n=10), which were inoculated and then treated with herbal extract at doses of 50 or 100 mg/kg of egg weight, respectively. At Hamburger Hamilton (HH) developmental stage 2224 (four days post-incubation), a 4 mm^2 aperture was created in the shell for imaging purposes. The Hamburger and Hamilton developmental phases of embryos were identified as previously demonstrated (Morariu, Briciu et al. 2025; Subbaraj et al. 2023). Images of the finest quality were generated utilizing a stereomicroscope (Luxeo 4D, CA, USA) and stored as TIF files.

Results

Authentication And Identification of Plants

Curcuma longa (Turmeric), *Zingiber officinale* (Ginger), *Ocimum sanctum* (Tulsi), and *Aloe barbadensis Miller* (Aloe Vera) were verified by the Botanical Survey of India, Howrah. Authentication and identification numbers include CNH/Tech.II/2025/1, CNH/Tech.II/2025/5, CNH/Tech.II/2025/8, and CNH/Tech.II/2025/7.

Determination Of Extractive Value

After removing the solvent under reduced pressure, the *Curcuma longa* (Turmeric) extract yield value is 8.5%, *Zingiber officinale* (Ginger) extract yield value is 9.2%, *Ocimum sanctum* (Tulsi) yield value is 7.5%, and *Aloe barbadensis* Miller (Aloe Vera) extract yield value is 8.5%, respectively.

Phytochemical Screening

All the ethanol extracts of medicinal plants contained different phytocompounds, as shown in Table 1. Polar compounds are observed in a more positive form in comparison to non-polar compounds, as ethanol belongs to the polar class that is responsible for the extraction of polar compounds.

Table 1: Phytochemical Screening of Plant Extracts

Test	**Ethanolic Extract of *Curcuma longa***	**Ethanolic Extract of *Zingiber officinale***	**Ethanolic Extract of *Ocimum sanctum***	**Ethanolic Extract of *Aloe barbadensis***
Alkaloids	Positive	Positive	Positive	Positive
Flavonoids	Positive	Positive	Positive	Positive
Glycosides	Positive	Positive	Negative	Negative
Saponins	Positive	Positive	Positive	Positive
Carbohydrate	Positive	Negative	Negative	Positive
Tannins	Positive	Positive	Positive	Negative
Terpenoids	Positive	Positive	Negative	Negative

Anti-Angiogenic Effects by CAM Assay

The chick chorioallantoic membrane (CAM) assay was utilized to assess the anti-angiogenic properties of four herbal extracts: Curcuma longa (Turmeric), Zingiber officinale (Ginger), Ocimum sanctum (Tulsi), and Aloe barbadensis Miller (Aloe Vera). Table 2 and Figure 1 present a summary of the results. The anti-angiogenic effect of the control group was low, with an average of 0.10 ± 1.50, which implies

baseline angiogenesis without treatment. The anti-angiogenic potential assay showed the greatest efficacy of curcuma longa with mean values of 2.8 ± 0.17 at 50 mg/kg and 3.6 ± 0.20 at 100 mg/kg. It shows a dose-dependent effect, with a moderate to severe anti-angiogenic effect. Zingiber officinale had significant inhibitory activity on neovascularization with the scores of 2.5 ± 0.16 and 3.2 ± 0.18 at 50 and 100 mg/kg, respectively, meaning moderate and strong effects. Its inhibitory effect on Ocimum sanctum was intermediate, and the results were 1.5 ± 0.12 (weak) and 2.6 ± 0.15 (moderate) at the two doses used. Aloe barbadensis Miller showed the least inhibition in the treated groups with an average of 1.2 ± 0.10 in group 1, and 2.1 ± 0.13 in group 2, indicating weak to moderate response.

Table 2: Anti-angiogenic effects of different *Curcuma longa*, *Zingiber officinale*, *Ocimum sanctum*, and *Aloe barbadensis* Miller extracts

Extracts	**Dose(mg/kg)**	**Average score**	**Anti-angiogenic effect**
Control	-	0.10 ± 1.50	None
Curcuma longa	50	2.8 ± 0.17	Moderate to Strong
	100	3.6 ± 0.20	Strong
Zingiber officinale Roscoe	50	2.5 ± 0.16	Moderate
	100	3.2 ± 0.18	Strong
Ocimum sanctum	50	1.5 ± 0.12	Weak
	100	2.6 ± 0.15	Moderate
Aloe barbadensis Miller	50	1.2 ± 0.10	Weak
	100	2.1 ± 0.13	Moderate

Vascular Branching Pattern

The effect of Curcuma longa (Turmeric), Zingiber officinale (Ginger), Ocimum sanctum (Tulsi), and Aloe vera on anti-angiogenesis was tested by measuring vessel area, total vessel length, vascular branch number, and lacunarity in a dose-dependent test algorithm (50mg/kg and 100mg/kg). The control group exhibited the highest rates of all angiogenesis-related markers indicating that the blood vessels were resilient without therapy. The vessel area was 63.59 +1.43 (control group) in the control group. The area of the blood vessels was significantly reduced by all plant extracts, particularly at 100 mg/kg dosage. The most significant inhibition was observed with Turmeric (100 mg/kg, 38.50 ± 1.20%, $p < 0.001$), followed by Tulsi (100 mg/kg, 39.45 ± 1.75%, $p < 0.001$), and subsequently Ginger (100 mg/kg, 41.80 ± 1.40%, $p < 0.001$). Aloe Vera 100 mg/kg was shown to reduce moderately yet significantly (51.90 ± 1.50%, $p < 0.01$). Reduced dosage produced a less pronounced effect. Tulsi at 50 mg/kg recorded a slight decrease ($p < 0.05$), whereas Aloe Vera at 50mg/kg did not show a statistical significant change (Table 3). The control group displayed a total vessel length of 8725.32 ± 1.87 pixels. Each treatment group demonstrated a significant, dose-dependent reduction. Turmeric at 100 mg/kg exhibited the most pronounced reduction (4300.55 ± 2.00*, $p < 0.001$), followed by Tulsi at 100 mg/kg (4724.37 ± 2.07*, $p < 0.001$), and subsequently Ginger at 100 mg/kg (4900.30 ± 2.25*, $p < 0.001$). Aloe Vera at 100 mg/kg produced a substantial decrease (6850.00 ± 2.10, $p < 0.01$). There were statistically significant results in the administration of 50 mg/kg of Turmeric and Ginger ($p < 0.01$), but there were no significant differences in Tulsi 50mg/kg and Aloe Vera 50mg/kg (Table 3).

Table 3: Vascular branching pattern analysis of different *Curcuma longa*, *Zingiber officinale*, *Ocimum sanctum*, and *Aloe barbadensis* Miller extracts

Groups	**Dose (mg/kg)**	**Parameters**			
		Vessel's area (%)	**Total vessels length (pixel)**	**Vascular branch**	**Lacunarity**
Control	-	63.59 ±1.43	8725.32 ±1.87	133± 4.25	0.34±0.03
Curcuma longa	50	52.80 ± 1.35****	7050.20 ± 2.10***	95 ± 3.50***	0.47 ± 0.05*
	100	38.50 ± 1.20****	4300.55 ± 2.00***	48 ± 3.00***	0.98 ± 0.06****
Zingiber	50	55.20 ±	7400.40 ±	108 ± 3.10*	0.42 ± 0.04**

officinale Roscoe		1.28**	2.30**	*	
	100	41.80 ± 1.40***	4900.30 ± 2.25***	62 ± 3.25***	0.82 ± 0.05***
Ocimum sanctum	50	57.56 ± 1.51*	8546.04 ± 3.49(ns)	127 ± 2.93(ns)	0.36 ± 0.03(ns)
	100	39.45 ± 1.75***	4724.37 ± 2.07***	50 ± 3.96***	0.92 ± 0.21***
Aloe barbadensis Miller	50	60.10 ± 1.36(ns)	8350.10 ± 2.20(ns)	126 ± 3.10(ns)	0.36 ± 0.03(ns)
	100	51.90 ± 1.50**	6850.00 ± 2.10**	88 ± 3.50**	0.51 ± 0.04*

Values are presented as the mean ± standard error of the mean. (ns) = Not statistically significant ($p > 0.05$). Asterisks indicate significance compared to control group values.

The control group was characterized by high complexity of the vascularity, with a number of 133 ± 4.25 branches observed. Significant reductions were observed with Turmeric 100 mg/kg (48 ± 3.00*, $p < 0.001$), Tulsi 100 mg/kg (50 ± 3.96*, $p < 0.001$), and Ginger 100 mg/kg (62 ± 3.25*, $p < 0.001$). Aloe Vera had a smaller, yet noteworthy effect with a dosage of 100 mg/kg (88 ± 3.50, $p < 0.01$). At lower doses (50 mg/kg), Turmeric and Ginger had a significant effect on reducing the branching, but Tulsi and Aloe Vera did not show significant changes compared to the control (Table 3).

The variability of the vascular pattern, known as lacunarity, increased with treatments, indicating that the vascular structure grew increasingly irregular and chaotic. The control group exhibited a lacunarity of 0.34 ± 0.03, which indicates a homogenous network. A significant increase was noted in Turmeric 100 mg/kg (0.98 ± 0.06*), Tulsi 100 mg/kg (0.92 ± 0.21*), and Ginger 100 mg/kg (0.82 ± 0.05*). Aloe Vera induced a modest increase (0.51 ± 0.04, $p < 0.05$). The 50 mg/kg dose of Turmeric and Ginger also showed significant median increases ($p < 0.05$ -0.01). Aloe Vera 50mg/kg and Tulsi 50mg/kg did not display any significant difference compared to the control (Table 3).

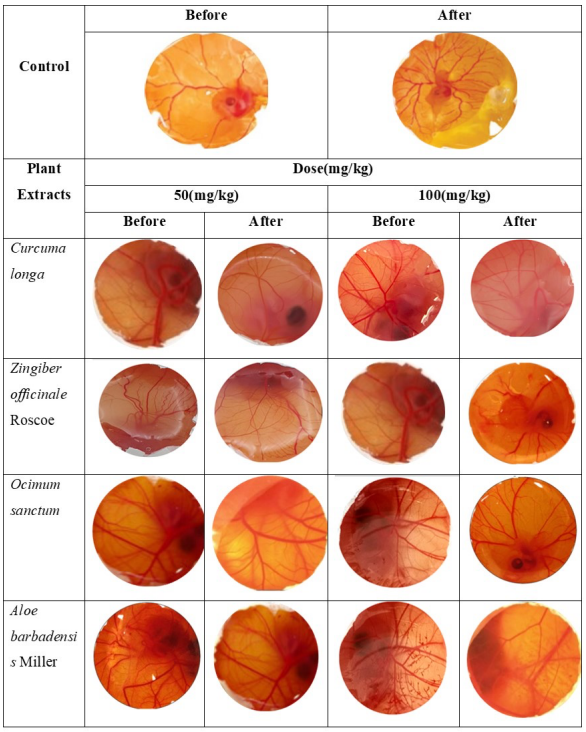

Figure 1: Images of live CAM implanted with Turmeric, Ginger, Tulsi and Aloe Vera at different doses. DMSO was used as a negative control, in which no significant inhibition of angiogenesis was observed. The concentration of 100 mg/ml showed good angiogenesis inhibition in Turmeric and Ginger as compared to Tulsi and Aloe Vera.

Discussion

The results show that all the plant extracts suppressed angiogenesis in a dose-dependent fashion, and Curcuma longa revealed the strongest activity in all of the parameters considered. Angiogenesis is a highly controlled physiological process, which has been exploited in a number of pathological conditions, particularly cancer, in which the process facilitates the proliferation and metastasis of tumors. According to Ribatti (2008), the ability of tumor growth to attain a certain size relies on angiogenesis. In this regard, blocking the angiogenic pathway has been found to be an important method of anti-cancer therapy. This hypothesis is supported by the results of this study, where the studied phytochemicals significantly decreased the growth of vessels relative to the unprotected control (Ribatti 2008).

Curcuma longa had the highest mean scores (3.6 ± 0.20 at 100 mg/kg), which indicates a better anti-angiogenic activity of Curcuma longa over all the tested extracts. It revealed significant reductions in vessel area, total vessel length, and vascular

branching, and a significant increase in lacunarity ($p < 0.001$). Such effects are presumably attributed to curcumin, the main bioactive compound of turmeric, which has been reported to inhibit endothelial cell proliferation and angiogenic signal transduction, therefore suppressing VEGF, bFGF, and NF-KB (Astinfeshan et al. 2019; Wang and Chen 2019). Zingiber officinale demonstrated significant anti-angiogenic activities, with the most significant effect being observed at 100mg/kg dosage (mean CAM score: 3.2 ± 0.18). Similar to turmeric, ginger had a strong influence on reducing all vascularity indices and improving lacunarity. Earlier studies have consolidated those same effects, showing that the main pungent compound of ginger is [6]-gingerol, which inhibits angiogenesis by decreasing MMP-9 and VEGFR2 signaling pathways. (Jiang, 2025). Though slightly less effective than turmeric, it justifies its inclusion under the banner of plausible natural anti-angiogenic agents. Ocimum sanctum showed medium inhibition at the dosage of 100 mg/kg (score: 2.6 ± 0.15), leading to a significant decrease of the vessel area and vascular branching. However, the effect at 50 mg/kg was less noticeable, and slight differences were noted in vascular parameters. The observed activity can be linked to the presence of the phytoconstituents eugenol and ursolic acid, both of which have reported anti-inflammatory and anti-angiogenic properties (Arulnangai et al. 2025). The evidence indicates that higher doses of tulsi extract are required to produce therapeutic anti-angiogenic effects. Aloe barbadensis Miller recorded the lowest anti-angiogenic effect, with CAM scores of 1.20 ± 0.10 (weak) at 50mg/kg and 2.10 ± 0.13 (moderate) at 100mg/kg. Aloe vera has known therapeutic and antioxidant properties; however, its direct anti-angiogenic activity on angiogenesis appears to be unique to aloe vera (Kaewsrisung et al. 2021). However, even at the higher doses, vascular measures slightly decreased, indicating that the drug was not quite effective.

Quantitative image analysis supported the CAM ratings, giving objective measurements of angiogenesis. Lacunarity was the sensitive indicator of modified vascular patterning, particularly in the turmeric- and ginger-treated groups. Increased lacunarity is an indication of disproportionate and scarce vascular networks, an indicator of impaired angiogenesis (Lungu et al. 2025). A significant strength of the study is the use of a validated in vivo model (CAM test), which includes drug administration and multi-parametric analysis. The major limitation, though, is the lack of molecular validation (i.e., gene or protein expression of VEGF, MMPs). Further studies need to employ immunohistochemistry or transcriptomic methods to clarify the particular molecular processes that may be participating in the process.

Conclusion

This paper demonstrates that Curcuma longa and Zingiber officinale have the potential to inhibit angiogenesis using natural inhibitors, which affect neovascularization dose-dependently. Ocimum sanctum showed moderate activity, and Aloe barbadensis Miller displayed weak activity. The results suggest the feasibility of employing plant-derived chemicals to augment or substitute other therapy in angiogenesis-driven disorders, including cancer. Subsequent molecular investigations should confirm these results to clarify the fundamental mechanisms.

Funding

None.

Conflict of Statement

The authors declare that they have no financial or non-financial competing interests related to this manuscript.

Data Availability

The data will be available upon reasonable request from the authors.

Declarations

Ethics Approval and Consent to Participate

Not Applicable

Consent for Publication

Not applicable

Acknowledgments

The authors are grateful to Chettinad Hospital and Research Institute and Chettinad Academy of Research and Education for their support.

Author Contributions

Conceptualization, Methodology, Writing-original draft: Umme Habiba, Debdip Mandal, and Anirban Karmakar; **Resources**, **Software, Visualization:** Mohini Mondal, Biplab Debnath, Joy Das and Sathvik Belagodu Sridhar; **Data curation**, **Investigation, Writing-original draft:** Uttam Prasad Panigrahy, Shubhrajit Mantry, and Vijay Singh; **Formal analysis, Project administration, Supervision, Writing-review and editing:** Rounak Seal

References

Arulnangai R, Asia Thabassoom H, Vajiha Banu H, et al. (2025) Recent developments on ursolic acid and its potential biological applications.

Toxicol Rep 14: 101900.10.1016/j.toxrep.2025.101900

Arya R, Faruquee HM, Shakya H, et al. (2024) Harnessing the Antibacterial, Anti-Diabetic and Anti-Carcinogenic Properties of Ocimum sanctum Linn (Tulsi). *Plants (Basel)* 13(24).10.3390/plants13243516

Astinfeshan M, Rasmi Y, Kheradmand F, et al. (2019) Curcumin inhibits angiogenesis in endothelial cells using downregulation of the PI3K/Akt signaling pathway. *Food Bioscience* 29: 86-93

Bhoopathy J, Vedakumari Sathyaraj W, Yesudhason BV, et al. (2024) Haemostatic potency of sodium alginate/aloe vera/sericin composite scaffolds–preparation, characterisation, and evaluation. *Artificial Cells, Nanomedicine, and Biotechnology* 52(1): 35-45

Chaudhary P and Janmeda P (2023) Comparative pharmacognostical standardization of different parts of Euphorbia neriifolia Linn. *Vegetos* 36(1): 210-221

Chaudhary P, Janmeda P, Pareek A, et al. (2024) Etiology of lung carcinoma and treatment through medicinal plants, marine plants and green synthesized nanoparticles: A comprehensive review. *Biomedicine & Pharmacotherapy* 173: 116294

Chaudhary P, Varshney N, Singh D, et al. (2022) Analysis of pharmacognostical standardization, antioxidant capacity and separation of phytocompounds from five different vegetable peels using different solvents. *Environment Conservation Journal* 23(3): 247-259

Fuloria S, Mehta J, Chandel A, et al. (2022) A Comprehensive Review on the Therapeutic Potential of Curcuma longa Linn. in Relation to its Major Active Constituent Curcumin. *Front Pharmacol* 13: 820806.10.3389/fphar.2022.820806

Galvão AM, Ferreira-Dias G and Skarzynski DJ (2013) Cytokines and angiogenesis in the corpus luteum. *Mediators Inflamm* 2013: 420186.10.1155/2013/420186

Iranmanesh M, Mohebbati R, Forouzanfar F, et al. (2018) In vivo and In vitro effects of ethanolic extract of Trigonella foenum-graecum L. seeds on proliferation, angiogenesis and tube formation of endothelial cells. *Res Pharm Sci* 13(4): 343-352.10.4103/1735-5362.235161

Jiang X (2025) Biological mechanisms, pharmacological and pathological activities, and quality optimization of gingerols and shogaols. *Journal of Functional Foods* 127: 106773

Kaewsrisung S, Sukpat S, Issarasena N, et al. (2021) The effects of oral Aloe vera on the efficacy of transplanted human endothelial cells and the expression of matrix metalloproteinases in diabetic wound healing. *Heliyon* 7(12)

Kaur S and Bains K (2024) Aloe Barbadensis Miller (Aloe Vera). *Int J Vitam Nutr Res* 94(3-4): 308-321.10.1024/0300-9831/a000797

Liu X, Zhang J, Yi T, et al. (2025) Decoding tumor angiogenesis: pathways, mechanisms, and future directions in anti-cancer strategies. *Biomarker Research* 13(1): 62

Lorenc P, Sikorska A, Molenda S, et al. (2024) Physiological and tumor-associated angiogenesis: Key factors and therapy targeting VEGF/VEGFR pathway. *Biomedicine & Pharmacotherapy* 180: 117585

Lugano R, Ramachandran M and Dimberg A (2020) Tumor angiogenesis: causes, consequences, challenges and opportunities. *Cell Mol Life Sci* 77(9): 1745-1770.10.1007/s00018-019-03351-7

Lungu CN, Mangalagiu II, Romila A, et al. (2025) Molecular Mediated Angiogenesis and Vasculogenesis Networks. *International Journal of Molecular Sciences* 26(13): 6316

Mao QQ, Xu XY, Cao SY, et al. (2019) Bioactive Compounds and Bioactivities of Ginger (Zingiber officinale Roscoe). *Foods* 8(6).10.3390/foods8060185

Marshall KM, Kanczler JM and Oreffo RO (2020) Evolving applications of the egg: chorioallantoic membrane assay and ex vivo organotypic culture of materials for bone tissue engineering. *J Tissue Eng* 11: 2041731420942734.10.1177/2041731420942734

Morariu-Briciu DM, Bolintineanu SL, Rata A, et al. (2025) Assessment of the biosafety profile of Galium verum in vitro on myoblasts and in ovo on chorioallantoic membrane. *World J Cardiol* 17(2): 102310.10.4330/wjc.v17.i2.102310

Oktavia S, Wijayanti N and Retnoaji B (2017) Anti-angiogenic effect of Artocarpus heterophyllus seed methanolic extract in ex

ovo chicken chorioallantoic membrane. *Asian Pacific Journal of Tropical Biomedicine* 7(3): 240-244

Pattanayak P, Behera P, Das D, et al. (2010) Ocimum sanctum Linn. A reservoir plant for therapeutic applications: An overview. *Pharmacogn Rev* 4(7): 95-105.10.4103/0973-7847.65323

Rawat M, Chaudhary P, Tulika K, et al. (2025) Pharmacognostical standardization, antioxidant capability, and phytocompound separation from fruit peels. *Environment Conservation Journal* 26(3)

Ribatti D (2008) Judah Folkman, a pioneer in the study of angiogenesis. *Angiogenesis* 11(1): 3-10.10.1007/s10456-008-9092-6

Ribatti D, Vacca A, Nico B, et al. (2003) Angiogenesis: basic and clinical aspects. *Ital J Anat Embryol* 108(1): 1-24

Saman H, Raza SS, Uddin S, et al. (2020) Inducing Angiogenesis, a Key Step in Cancer Vascularization, and Treatment Approaches. *Cancers (Basel)* 12(5).10.3390/cancers12051172

Sánchez M, González-Burgos E, Iglesias I, et al. (2020) Pharmacological Update Properties of Aloe Vera and its Major Active Constituents. *Molecules* 25(6).10.3390/molecules25061324

Sharifi-Rad J, Rayess YE, Rizk AA, et al. (2020) Turmeric and Its Major Compound Curcumin on Health: Bioactive Effects and Safety Profiles for Food, Pharmaceutical, Biotechnological and Medicinal Applications. *Front Pharmacol* 11: 01021.10.3389/fphar.2020.01021

Sharma S, Shukla MK, Sharma KC, et al. (2023) Revisiting the therapeutic potential of gingerols against different pharmacological activities. *Naunyn Schmiedebergs Arch Pharmacol* 396(4): 633-647.10.1007/s00210-022-02372-7

Sofowora A, Ogunbodede E and Onayade A (2013) The role and place of medicinal plants in the strategies for disease prevention. *Afr J Tradit Complement Altern Med* 10(5): 210-229.10.4314/ajtcam.v10i5.2

Subbaraj GK, Masoodi T, Yasam SK, et al. (2023) Anti-angiogenic effect of nano-formulated water soluble kaempferol and combretastatin in an in vivo chick chorioallantoic membrane model and HUVEC cells. *Biomed Pharmacother* 163: 114820.10.1016/j.biopha.2023.114820

Wang TY and Chen JX (2019) Effects of Curcumin on Vessel Formation Insight into the Pro- and Antiangiogenesis of Curcumin. *Evid Based Complement Alternat Med* 2019: 1390795.10.1155/2019/1390795

Zahra FT, Sajib MS and Mikelis CM (2021) Role of bFGF in Acquired Resistance upon Anti-VEGF Therapy in Cancer. *Cancers (Basel)* 13(6).10.3390/cancers13061422