International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 11, Issue 5, pp: 215-225, 2026
https://doi.org/10.55126/ijzab.2026.v11.i05.028 **http://www.ijzab.com**

Research Article

EMBRYOTOXICITY AND ANTIANGIOGENIC POTENTIAL OF THE AQUEOUS BARK EXTRACT OF THE ENDEMIC PHILIPPINE CINNAMON *CINNAMOMUM MINDANAENSE* IN MALLARD DUCK EMBRYOS USING THE CHORIOALLANTOIC MEMBRANE ASSAY

Jay P. Picardal

Gums, Resins, Oils and Woodcraft Technology Hub (G.R.O.W.T.H.) Laboratory Research Institute for Tropical Biology and Pharmacological Biotechnology, Cebu Normal University, Osmeña Boulevard, Cebu City, Philippines

Article History: Received 27th May 2026; Accepted 28th July 2026; Published 30th September 2026

ABSTRACT

Angiogenesis is a regulated process necessary for normal embryonic development, blood vessel growth, and tissue repair. Among Philippine plants, little is known about the antiangiogenic potential of Philippine endemic cinnamon. This study evaluated the embryotoxicity and antiangiogenic potential of the aqueous bark extract of *C. mindanaense* using the mallard duck (*Anas platyrhynchos*) chorioallantoic membrane (CAM) model. Fertilized eggs (N=40, 1 day old) were randomly assigned to an untreated control, solvent control (ddH_2O), positive control (retinoic acid), and five concentrations of aqueous bark extract (100%, 50%, 25%, 12.5%, and 6.25%). Embryonic development was evaluated by measuring crown-rump, forelimb, hindlimb, and beak. The complexity of vascular branching was quantified by the Fractal Dimension Index (FDI) derived from ImageJ analysis of CAM images. All morphological parameters were significantly affected by the experimental treatment ($P < 0.05$). The maximum reduction in crown-to-rump and forelimb length in the extract-treated groups was observed with 100% aqueous bark extract, whereas lower concentrations exhibited morphometric measurements almost similar to the controls. The degree of branching of the vascular network differed significantly between treatments (fractal analysis; Kruskal–Wallis H (7) = 31.20, $P < 0.001$, $\varepsilon^2 = 0.756$). The minimum FDI was obtained with 100% aqueous bark extract in the embryos, which was 26.2% lower than the untreated control, and the FDI increased with the dilution of the extract. The aqueous bark extract of *C. mindanaense* exhibited concentration-dependent embryotoxic and antiangiogenic effects, with the undiluted extract producing the greatest inhibition of embryonic growth and CAM vascular branching. These findings identify *C. mindanaense* as a promising endemic Philippine species for future studies on plant-derived antiangiogenic compounds.

Keywords: Angiogenesis, Embryotoxicity, Chorioallantoic membrane assay, Philippine endemic cinnamon.

INTRODUCTION

Angiogenesis is a multistep physiological development where new blood vessels are formed from pre-existing blood vessels. Under neovascularization, angiogenesis follows vasculogenesis, and both are widely recognized processes that support embryonic development, tissue repair, wound healing, and reproduction (Deryugina and Quigley, 2008; Irvin *et al*., 2014). During normal embryonic growth, angiogenesis ensures the continuous supply of oxygen and nutrients needed for organ formation through the coordinated action of signaling molecules such as vascular endothelial growth factor (VEGF). However, abnormal angiogenesis is associated with the development and progression of several diseases, including cancer (Carmeliet & Jain, 2011; Lugano *et al*., 2020; Yang *et al*., 2024). While antiangiogenic drugs have improved the treatment of many of these conditions, their long-term use is often limited by drug resistance, adverse effects, toxicity, and high cost. These limitations have encouraged the search for plant-derived compounds that can serve as safer and more affordable antiangiogenic agents (Hoseinkhani *et al.*, 2020; Abu-Reidah & Taamalli, 2024). Among the

*Corresponding Author: Jay P. Picardal, (G.R.O.W.T.H.) Laboratory Research Institute for Tropical Biology and Pharmacological Biotechnology Cebu Normal University, Osmeña Boulevard, Cebu City, Philippines Email: picardalj@cnu.edu.ph

Philippine medicinal plants reported to date, cinnamon possesses anti-angiogenic potential linked to the genus' rich composition of major phytochemicals such as cinnamaldehyde, eugenol, cinnamic acid derivatives, flavonoids, tannins, and essential oils that have been shown to inhibit endothelial cell proliferation, suppress VEGF-mediated signaling, and interfere with tumor angiogenesis (Rao & Gan, 2014; Hoseinkhani *et al*., 2020). Empirical findings from other studies show that Indian cinnamon (*C. tamala*) and Sri Lankan cinnamon (*C. zeylanicum*) both blocked tumor growth by inhibiting angiogenesis (Thanekar e*t al*., 2016; Zhang *et al*., 2009). Chinese cinnamon (*C. cassia*) also inhibited angiogenesis in zebrafish and human umbilical vein endothelial cell assays via VEGFR1 and VEGFR2 inhibition (Bansode *et al*., 2012). In a more recent study, one of the 16 Philippine endemic cinnamon species (*C. cebuense*) has been found to possess a strong antiangiogenic potential when administered in high concentration (100% w/v) (Alimpoos *et al*., 2018). This conclusion drew support from a previously published paper on *C. cebuense,* where a specific compound under the eugenol family (i.e., 4-allyl-2-methoxyphenol) was isolated from the dichloromethane extract of air-dried bark of *C. cebuense* (Ragasa *et al*., 2013). In a previous assay, 4-allyl-2-methoxyphenol was reported to be cytotoxic against HL-60 leukemia cells with a 50% cytotoxic concentration (CC50) of 0.38 mM (Hirata *et al*., 2005). Eugenol has been reported to suppress angiogenesis by reducing the expression of VEGF and VEGF receptor 1, as well as inhibiting matrix metalloproteinases involved in vascular invasion (Manikandan *et al*., 2010). Interestingly, *C. cebuense* root and bark decoction has been reported to be drunk by local communities in Cebu so as to relieve gastrointestinal problems (Del Fierro *et al*., 2012; Alimpoos *et al*., 2018).

Despite extensive investigations on commercially important species such as *Cinnamomum verum* (Sri Lanka), *C. cassia* (China), and *C. burmannii* (Indonesia), other endemic Philippine cinnamon species remain poorly explored in terms of biological activity. One such species, *Cinnamomum mindanaense*, has been traditionally used for treating stomach ulcers, headaches, diabetes, and wound infections. However, there is no published data related to its embryotoxicity or antiangiogenic activity. Since compounds capable of suppressing angiogenesis may also interfere with normal embryonic vascular development, simultaneous assessment of embryotoxicity and vascular inhibition is essential during early pharmacological screening (Ribatti, 2022; Almatroudi *et al*., 2024). Thus, this present paper hypothesizes that increasing extract concentrations would differentially influence embryonic morphogenesis and vascular development, with higher concentrations producing greater inhibition of angiogenesis. Testing this hypothesis through *in vitro* CAM assay may provide baseline information for future phytochemical characterization, molecular investigations, and natural-product drug discovery of this widely-used and popular endemic cinnamon species in the Philippines.

MATERIALS AND METHODS

Study Site and Experimental Design

The study was conducted at the Biology Laboratory of Cebu Normal University, Cebu City, Philippines, where all laboratory procedures, embryo incubation, morphological assessment, and chorioallantoic membrane (CAM) analyses were performed under ambient laboratory conditions. The experiment employed a completely randomized design (CRD) consisting of three control groups: untreated control, solvent control (double distilled water) and positive control (retinoic acid). Under the untreated control, eggs were maintained under identical incubation conditions without administration of any treatment to represent normal embryonic development; solvent control used distilled water (0.1 ml) injected into the air space while in positive control groups, embryos received 0.1 ml of analytical-grade retinoic acid, a well-established teratogenic compound and angiogenesis inhibitor. For the experimental treatment groups, embryos' air space was administered with 0.1 mL of aqueous *C. mindanaense* bark extract at the following concentrations: T1=100%, T2=50%, T3=25%, T4=12.5%, and T5=6.25%. All treatments were represented by 5 mallard duck egg (N=40; 1d old fertilized egg) samples. Both control and experimental groups were randomly administered the assigned concentration, respectively.

Plant Collection and Authentication

The inner bark of *Cinnamomum mindanaense* was purchased from cinnamon farmers in Barangay San Antonio, Boljoon, Cebu, the Philippines. The area (9.6739° N, 123.4129° E) has abundant natural stands of the Cinnamomum *mindanaense* species and has been an alternative source of livelihood for the vegetable farmers in the locality. Plant identification and authentication were based on standard taxonomic characters following Philippine floristic references (Kostermans, 1986), citing fruit size, cupula structure, and diagonal secondary veins as dominant diagnostic characters. In the field, bark samples were collected from mature individuals (with branches around 12 months – 18 months old) to minimize variation associated with developmental stage or phenotypic plasticity. Following the standard protocol for bark harvesting, this paper adopted the Sri Lankan approach of removing the knots, scraping the katta, rubbing the inner bark with brass, delamination using a knife, and drying of the inner bark under indirect sunlight for 72 h. Voucher specimens (rachis with dried inner bark) were deposited and taxonomic identification was done at the CNU Herbarium.

Preparation of the Aqueous Bark Extract

The aqueous bark extract was prepared using a decoction method following the traditional preparation observed in

Philippine ethnomedicinal practice. Briefly, 50 grams of dried inner bark of *C. mindanaense* were soaked and boiled gently in 1,000 mL of distilled water for 30 min. The resulting decoction was cooled to room temperature and filtered through Whatman No. 1 filter paper to remove plant debris. The filtrate represented the 100% stock extract. Using sterile distilled water as the diluent, two-fold serial dilution was subsequently performed to obtain extract concentrations of 50%, 25%, 12.5%, and 6.25%. All prepared solutions were kept in an airtight amber bottle and stored in the refrigerator until use within a 12h period.

Experimental Duck Eggs

Forty (40) freshly fertilized mallard duck eggs (one day post-laying, ~65-75 g weight) were obtained from a commercial duck breeder in Carcar City, Cebu, Philippines. The eggs were cleaned using sterile tissue paper to remove adhering debris before candling to confirm fertilization. Fertilized eggs exhibiting a distinct germinal disc were included in the experiment, whereas unfertilized eggs or eggs with visible shell defects were excluded. Eggs were incubated in a laboratory incubator with the following parameters: temperature of 37.5 ± 0.3°C and relative humidity of 50-55%. Eggs were manually turned at least twice daily (0900 h and 1600 h) until day 10 of incubation.

Administration of Experimental Treatments

On the 11th day of incubation, a small opening (5 mm^2) was carefully created over the air sac using a sterile needle under aseptic conditions within a laminar flow cabinet. Each embryo received 0.1 mL of the designated treatment through sterile tuberculin syringes. After inoculation, the shell opening was immediately sealed using sterile medical adhesive tape to prevent microbial contamination and moisture loss. Following treatment administration, eggs were returned to the incubator and maintained until Day 17 of embryonic development.

Morphological Assessment of Embryos

At Day 17, embryos were carefully removed from the eggshells and examined macroscopically. The following morphometric parameters were measured using a digital Vernier caliper: crown–rump length (mm), forelimb length (mm), hindlimb length (mm), and beak length (mm). Embryos were also examined for external developmental abnormalities, including body curvature, limb deformities, beak malformation, growth retardation, hemorrhage, and embryonic mortality. All measurements were recorded to the nearest 0.01 mm, and the basis of duck embryonic development referred to Hamburger and Hamilton (1951).

Chorioallantoic Membrane (CAM) Assay

Following embryo removal, the CAM was carefully separated from the eggshell and mounted flat on Whatman filter paper. Digital images were acquired immediately using a high-resolution camera (Nikon D3400) positioned at a fixed distance (10 inches from the laboratory table) to maintain image consistency among samples. The CAM assay served as an in vivo model for evaluating vascular branching complexity and angiogenic responses following treatment with *C. mindanaense* bark extract.

Image Acquisition and Fractal Dimension Analysis

Digital images of the CAM were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Images were converted to grayscale and subjected to standardized thresholding prior to analysis. Vascular complexity was quantified using the fractal box-counting algorithm implemented in ImageJ. The fractal dimension (FD) was calculated for each CAM image as an objective indicator of vascular branching complexity. Lower FD values were interpreted as reduced vascular branching complexity, suggesting potential antiangiogenic activity, whereas higher FD values indicated more extensive vascular development.

Statistical Analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA) to compare morphometric measurements and fractal dimension values among treatment groups. Prior to using ANOVA, several assumptions were met to qualify for parametric testing of independent groups. When significant differences were detected, Tukey's Honest Significant Difference (HSD) post hoc test was used for pairwise comparisons. Data are presented as mean ± standard deviation (SD). Statistical significance was established at $p < 0.05$. Meanwhile, Fractal Dimension Index (FDI) values generated using the ImageJ box-counting method were used to quantify the complexity of the chorioallantoic membrane (CAM) vascular branching pattern. Normality was assessed using the Shapiro–Wilk test, and because the data were not normally distributed, differences among treatment groups were analyzed using the Kruskal–Wallis test followed by Dunn's multiple-comparison test with Holm adjustment ($P < 0.05$). The magnitude of the treatment effect was estimated using epsilon-squared (ε^2). FDI values are presented as mean ± standard deviation (SD), while box-and-whisker plots illustrate the median, interquartile range, minimum–maximum values, and individual embryo measurements. Treatment groups sharing the same superscript letter were not significantly different according to Dunn's test ($P < 0.05$). Lower FDI values indicate simpler vascular networks with reduced branching complexity, consistent with inhibition of angiogenesis (Parsons-Wingerter et al., 1998; Guidolin et al., 2004).

RESULTS AND DISCUSSION

Morphometric measurements of mallard duck (*Anas platyrhynchos*) embryos differed significantly among the experimental groups (Tables 1 and 2). One-way ANOVA revealed significant treatment effects on crown-to-rump length ($F_{7,23} = 8.92$, $P < 0.001$, $\eta^2 = 0.731$), forelimb length ($F_{7,23} = 5.26$, $P = 0.001$, $\eta^2 = 0.615$), hindlimb length ($F_{7,23} = 21.51$, $P < 0.001$, $\eta^2 = 0.867$), and beak length ($F_{7,23} =$

3.35, $P = 0.013$, $\eta^2 = 0.505$). The large effect sizes indicate that treatment accounted for a substantial proportion of the observed variation, particularly in hindlimb development. The untreated control exhibited the greatest crown-to-rump length (81.30 ± 1.91 mm), whereas embryos exposed to retinoic acid showed the lowest value (71.05 ± 3.32 mm). Similarly, forelimb length was significantly shorter in the positive control (24.50 ± 1.56 mm) than in the untreated control (30.80 ± 0.57 mm). Among the extract-treated embryos, the 100% aqueous bark extract produced the shortest crown-to-rump (73.03 ± 1.39 mm) and forelimb (26.45 ± 0.90 mm) measurements, while embryos receiving the 6.25% extract exhibited measurements comparable with the untreated control. Tukey's HSD test further showed that most lower extract concentrations shared statistical groupings with the controls (Table 1), indicating that growth inhibition became more evident only at the highest concentration.

Table 1. Morphometric parameters of mallard duck (*Anas platyrhynchos*) embryos across experimental treatment groups.

Treatment Groups	n	Crown-to-rump length (mm)	Forelimb length (mm)	Hindlimb length (mm)	Beak length (mm)
Untreated control (non-manipulated control)	4	81.30 ± 1.91^{a}	30.80 ± 0.57^{a}	34.92 ± 0.87^{bcd}	12.97 ± 0.46^{a}
Solvent control (double distilled water)	4	78.10 ± 2.48^{abc}	28.17 ± 1.50^{abc}	31.90 ± 1.57^{de}	12.23 ± 1.37^{a}
Positive Control (Retinoic acid)	2	71.05 ± 3.32^{d}	24.50 ± 1.56^{c}	28.95 ± 0.64^{e}	9.60 ± 0.14^{b}
100% aqueous extract	3	73.03 ± 1.39^{bd}	26.45 ± 0.90^{bc}	34.17 ± 0.51^{cde}	11.38 ± 0.56^{ab}
50% aqueous extract	4	75.18 ± 2.51^{bcd}	29.58 ± 1.53^{ab}	39.22 ± 2.27^{ab}	11.58 ± 0.27^{ab}
25% aqueous extract	4	77.72 ± 2.24^{abc}	28.34 ± 2.64^{abc}	42.73 ± 2.46^{a}	12.37 ± 0.65^{a}
12.5% aqueous extract	5	78.05 ± 2.08^{ac}	28.86 ± 1.66^{abc}	37.14 ± 2.32^{bc}	11.96 ± 1.06^{ab}
6.25% aqueous extract	5	80.35 ± 0.80^{a}	31.33 ± 1.96^{a}	41.36 ± 1.87^{a}	12.34 ± 1.14^{a}

Values are mean ± SD. Within a column, means sharing at least one superscript letter are not significantly different according to Tukey's HSD test ($\alpha = 0.05$). Zero measurements from non-viable embryos were excluded.

The decrease in crown-to-rump length (Table 1, Figure 1a) and forelimb lengths (Table 1, Figure 1b) at the highest extract concentration shows that concentrated aqueous bark extract of *Cinnamomum mindanaense* can interfere with normal embryonic development. The total body length is generally accepted as a reliable marker of developmental progress during avian embryogenesis, as it reflects the cumulative growth resulting from coordinated cell proliferation, differentiation and tissue expansion (Hamburger and Hamilton, 1951; Ribatti, 2017). The reductions seen in the present study, although less marked than those induced by retinoic acid, are in agreement with previous observations from another endemic Philippine cinnamon, *Cinnamomum cebuense*, where the undiluted leaf extracts inhibited chorioallantoic membrane vascularization and arrested embryonic development in mallard duck embryos (Alimpoos *et al.,* 2018). Inhibition of endothelial cell proliferation and VEGF-mediated angiogenesis by cinnamon-derived phytochemicals have also been shown to have similar developmental effects (Lu *et al*., 2010; Bansode *et al*., 2013). The reduced body size at higher extract concentration may be in part due to inhibition of angiogenesis, as embryonic growth requires the establishment of an adequate vascular network for oxygen and nutrient transport. However, the active compounds responsible for these effects in *C. mindanaense* are still unknown and need to be further investigated phytochemically. As expected, the embryos in the retinoic acid group (Table 1, Figures 1a-d) showed the greatest decrease in all morphometric parameters. Not all embryos respond in the same way to retinoic acid, even when given the same nominal dose. For example, 2 of the 5 egg samples under positive control survived, but have a marked developmental delay (i.e. lower Hamburger Hamilton stage). This is reflected in stunted growth, reduced crown-to-rump length, shortened limbs and lower body weight. Variability of the egg samples and responses to the teratogen (i.e. retinoic acid) may be due to dose received by individual embryo, time of exposure, biological variability and egg quality. Excess retinoic acid perturbs tightly regulated retinoid signaling pathways which regulate axial patterning, craniofacial morphogenesis, somitogenesis and limb development in vertebrate embryos (Griffith and Wiley, 1991; Stratford et al., 1996; Rhinn and Dollé, 2012; Feneck and Logan, 2020). The substantial reduction in crown-to-rump, forelimb, hindlimb and beak measurements thus supports the sensitivity of the duck embryo CAM assay for the detection of developmental toxicity. These measurements should be taken with caution however, as only two embryos survived in the positive-control group. They are in agreement with the known teratogenic effects of retinoic acid. Unlike crown-rump and forelimb lengths, the length of the hindlimb (Table 1, Figure 1c) did not decrease with increasing concentration. The highest hindlimb lengths were noted in the embryos treated with 25% and 6.25% aqueous extracts, while the least was noted

in the positive control. Concentrations of extract next to each other did not give a consistent trend and so this pattern is unlikely to represent a stimulation of limb growth. Rather, the variation we observed may reflect natural variation among surviving embryos, small variation in developmental stage at harvest, or variation introduced during morphometric measurement. Similar inconsistencies have been reported in avian developmental studies where appendicular growth does not always parallel total embryonic growth (Hamburger and Hamilton, 1951; Drake and Little, 1991). Therefore, hindlimb measurements must be interpreted in the light of embryo survival, external malformations and vascular development and not as an isolated indicator of embryotoxicity.

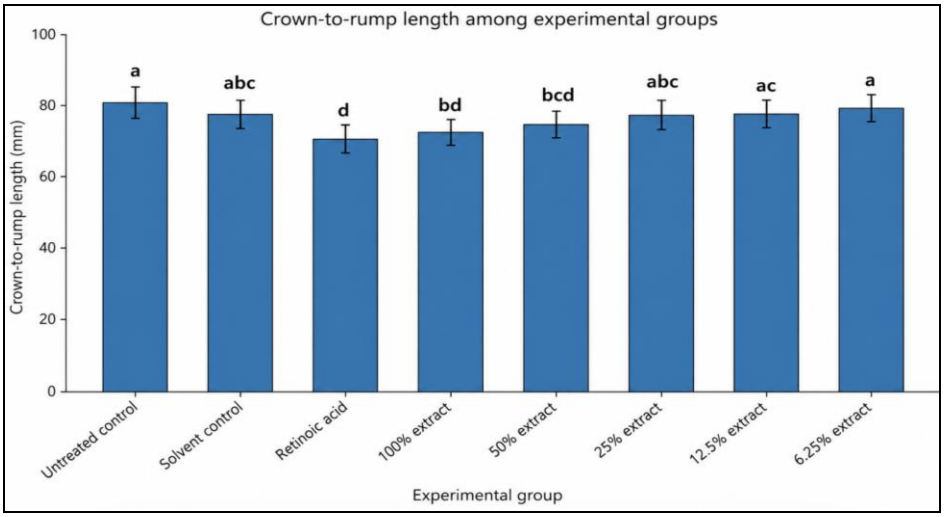

Figure 1a. Crown-to-rump length of mallard duck **(***Anas platyrhynchos***)** embryos following treatment with aqueous bark extract of *Cinnamomum mindanaense***.** Bars represent mean ± SEM. Different letters above the bars indicate significant differences among treatment groups according to Tukey's HSD test ($P < 0.05$).

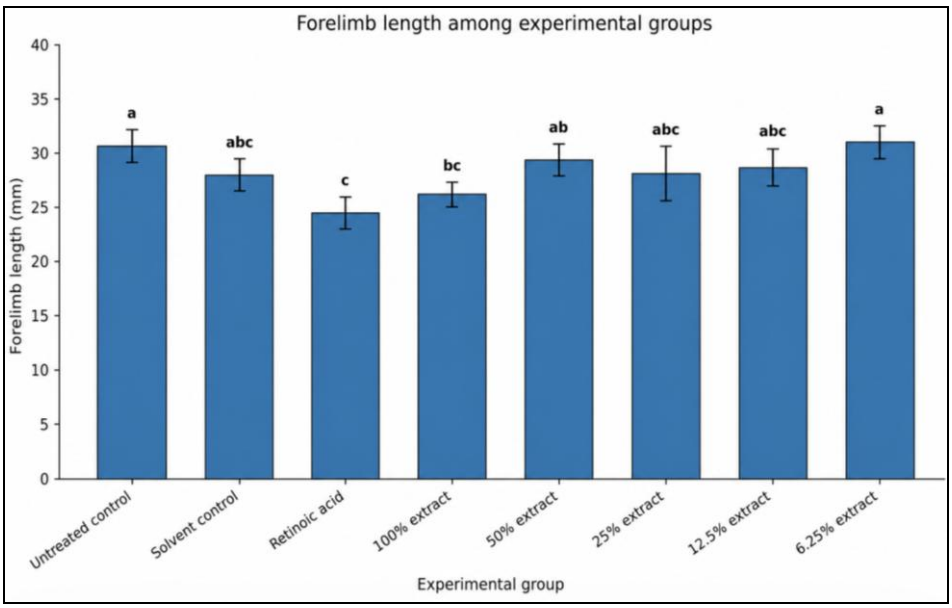

Figure 1b. Forelimb length of mallard duck **(***Anas platyrhynchos*) embryos following treatment with aqueous bark extract of *Cinnamomum mindanaense***.** Bars represent mean ± SEM. Different letters above the bars indicate significant differences among treatment groups according to Tukey's HSD test ($P < 0.05$).

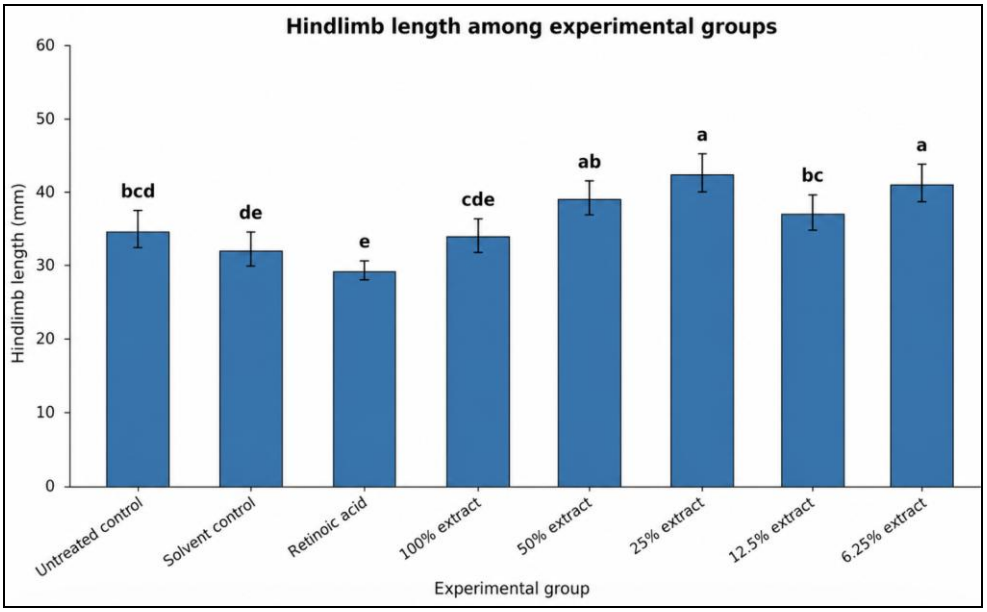

Figure 1c. Hindlimb length of mallard duck (*Anas platyrhynchos*) embryos following treatment with aqueous bark extract of *Cinnamomum mindanaense***.** Bars represent mean ± SEM. Different letters above the bars indicate significant differences among treatment groups according to Tukey's HSD test ($P < 0.05$)

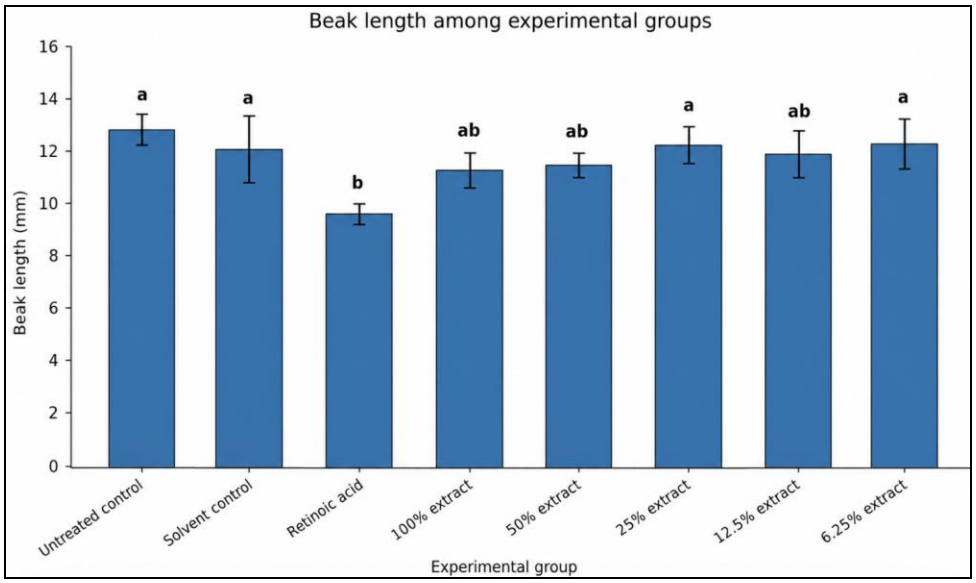

Figure 1d. Beak length of mallard duck (*Anas platyrhynchos*) embryos following treatment with aqueous bark extract of *Cinnamomum mindanaense***.** Bars represent mean ± SEM. Different letters above the bars indicate significant differences among treatment groups according to Tukey's HSD test ($P < 0.05$).

Beak length was the least affected morphometric parameter (Table 1, Figure 1d). Only the retinoic acid group was statistically clustered with the untreated and solvent controls, suggesting that the aqueous bark extract had relatively few effects on craniofacial growth under the experimental conditions. The formation of craniofacial structures involves complex interactions between neural crest cells, mesenchymal proliferation, and tissue-specific signaling pathways, which may be differentially sensitive to phytochemicals than rapidly developing axial and appendicular tissues (Maden, 2002; Rhinn and Dollé, 2012). The morphometric data overall suggest that the aqueous bark extract of *C. mindanaense* had observable developmental effects mainly at the highest concentration,

and that crown-to-rump and forelimb lengths were the most sensitive measures of embryonic growth inhibition. These results are in agreement with the angiogenesis data presented in this study and further support the investigation of *C. mindanaense* as a potential source of antiangiogenic compounds. Further phytochemical profiling, histopathological evaluation and molecular analyses of angiogenic regulators are required in future studies to elucidate the mechanisms behind the observed developmental responses. The fractal dimension index (FDI) differed significantly among the eight experimental groups (Kruskal–Wallis H (7) = 31.20, $P < 0.001$, $\varepsilon^2 = 0.756$). The effect size indicates that treatment accounted for a large proportion of the rank variation in vascular complexity. The untreated and solvent controls yielded mean FDI values of 1.768 ± 0.112 and 1.652 ± 0.062, respectively. The positive control treated with retinoic acid had an FDI of 0.000 ± 0.000, whereas the lowest measurable value among extract-treated groups occurred at 100% extract (1.305 ± 0.091). FDI increased as the preparation was diluted to 50%, 25%, and 12.5%, although the 6.25% group was slightly lower than the 12.5% group. Dunn's test with Holm correction separated the retinoic-acid group from the groups retaining measurable vascular networks and confirmed the marked reduction associated with the undiluted extract (Table 2).

Table 2. Fractal dimension index of the chorioallantoic membrane vascular network of mallard duck (*Anas platyrhynchos*) embryos following treatment with aqueous bark extract of *Cinnamomum mindanaense*.

Treatment Group	*n*	Fractal Dimension Index*
Untreated control	5	1.768 ± 0.112^{a}
Solvent control	5	1.652 ± 0.062^{abc}
Positive control—retinoic acid	5	0.000 ± 0.000^{c}
100% aqueous bark extract	5	1.305 ± 0.091^{bc}
50% aqueous bark extract	5	1.537 ± 0.070^{abc}
25% aqueous bark extract	5	1.681 ± 0.042^{abc}
12.5% aqueous bark extract	5	1.785 ± 0.069^{a}
6.25% aqueous bark extract	5	1.708 ± 0.107^{ab}

*Values are mean ± SD. Groups sharing at least one superscript letter are not significantly different according to Dunn's multiple-comparison test with Holm adjustment at $\alpha = 0.05$.

Table 3. Kruskal-Wallis Test of Fractal Dimension Index.

Dependent Variable	N	Groups	H	df	p	ε^{2}*	Interpretation
Fractal Dimension Index	40	8	31.197	7.000	0.000	0.756	Significant; large effect

*Effect size was calculated as $\varepsilon^2 = (H - k + 1)/(N - k)$. The rank-based $\eta^2 H$ estimate was 0.800.

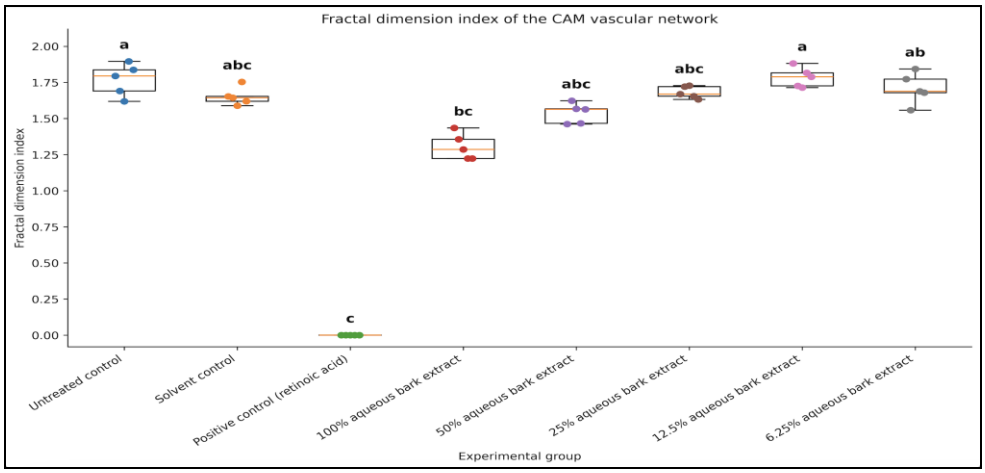

Figure 2. Fractal dimension index (FDI) of the chorioallantoic membrane (CAM) vascular network of mallard duck (*Anas platyrhynchos*) embryos following exposure to aqueous bark extract of *Cinnamomum mindanaense***.** The FDI was used as a

quantitative measure of vascular branching complexity. Box plots represent the median, interquartile range, and minimum–maximum values, with individual embryo measurements overlaid as dots. Different superscript letters indicate significant differences among treatment groups based on Dunn's multiple-comparison test with Holm adjustment ($p < 0.05$). Lower FDI values correspond to simpler vascular networks with reduced branching complexity, consistent with inhibition of angiogenesis.

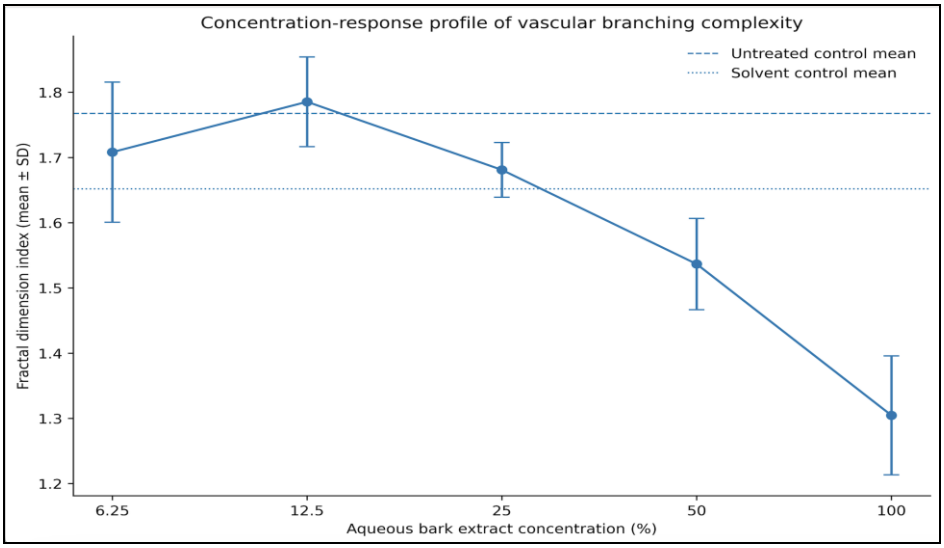

Figure 3. Concentration–response profile of the fractal dimension index (FDI) of the chorioallantoic membrane (CAM) vascular network in mallard duck **(***Anas platyrhynchos***)** embryos following treatment with aqueous bark extract of *Cinnamomum mindanaense***.** Values are expressed as mean ± SD (n = 5). Horizontal dashed and dotted lines indicate the mean FDI of the untreated and solvent controls, respectively. The overall trend shows a reduction in vascular branching complexity with increasing extract concentration, with the lowest FDI recorded in embryos treated with the 100% aqueous bark extract. This pattern supports a concentration-related inhibitory effect of the extract on CAM vascular development.

The zero FDI in all retinoic-acid-treated samples (Table 2) represents the lower limit of the scale used for image analysis and not a vascular complexity below zero. It may be reported as the lack of a measurable vascular network only if the images were successfully obtained, thresholded and analyzed using the same procedure applied to the other groups. If a CAM could not be assessed because an embryo died before this could occur, or if an image was blurry, incomplete or not suitable for segmentation, the observation should be considered missing. This distinction is important, since fractal estimates are sensitive to the amount and quality of information contained in the image. Recent reviews of methods based on fractal-dimension stress that the obtained estimate can be affected by noise, segmentation quality, resolution, scale selection, and methodological choices (Datseris et al., 2021).

The 100% aqueous bark extract reduced mean FDI by approximately 26.2% relative to the untreated control. This finding indicates substantial simplification of the CAM branching pattern at the highest extract concentration. The response is consistent with an earlier duck CAM study of the Philippine endemic *Cinnamomum cebuense,* which reported greater vascular inhibition at higher aqueous-extract concentrations (Alimpoos et al., 2018). It is also biologically plausible in view of experimental work showing that water-based cinnamon extracts can suppress VEGFR2 kinase activity and downstream MAPK/STAT3 signaling, and can inhibit angiogenesis in endothelial-cell and zebrafish models. The extract response was concentration-related but not strictly monotonic. The increase in FDI from 100% to 12.5% extract indicates that the inhibitory effect generally decreased with dilution. However, the response was not strictly monotonic, as evidenced by the slight dip at 6.25% relative to 12.5%. Additionally, the 12.5% treatment had the same statistical letter as the untreated control, and several intermediate treatments statistically overlapped with both the higher- and lower-FDI groups. The results thus indicate a trend related to concentration, but not a simple linear dose–response. Group means with only five embryos per group may also be affected by individual differences in developmental stage, CAM position, illumination, field selection and vessel visibility

Overall, the FDI findings provide the clearest evidence of vascular simplification in the retinoic-acid and 100% extract groups. The recovery of branching complexity after dilution suggests that the antiangiogenic effect of the aqueous bark preparation is strongest when undiluted. Chemical profiling and assays targeting VEGF-related signaling are needed before the effect can be attributed to a particular constituent or molecular pathway. Fractal dimension gives a measure of the degree of filling of the

image plane by a vascular pattern, which is scale-dependent. In a consistent set of CAM images acquired and processed consistently, lower FDI values suggest a less space-filling network with fewer or less extensive branches, and higher values indicate a more complex vascular arrangement. This interpretation is consistent with recent knowledge that angiogenesis is a dynamic process involving endothelial sprouting, branching, reconnection, pruning and remodeling, rather than a mere increase in the number of vessels. Similar to this, recent computational studies demonstrated that the architecture of vessels depends on endothelial-cell migration, bifurcation, connectivity, and rearrangement within developing sprouts (Mada and Tokihiro, 2021; Stepanova et al., 2023). The CAM is particularly suited for this type of analysis since its superficial and rapidly developing circulation can be observed and quantified without removing the vascular network from its living embryonic environment. Recent studies continue to characterize the CAM as a reproducible and biologically relevant in vivo platform for the study of vascular development, drug responses, molecular transport and tumour-associated angiogenesis (Mapanao et al., 2021; Schäfer et al., 2024). The present application of FDI thus supports the evaluation by substituting a purely visual description of vascular inhibition by a quantitative measure of network organization.

CONCLUSION

The aqueous bark extract of the Philippine endemic cinnamon *Cinnamomum mindanaense* showed concentration-dependent embryotoxic and antiangiogenic effects in the mallard duck chorioallantoic membrane assay, with the undiluted extract showing the greatest inhibition of embryonic growth and vascular branching complexity. This study provides the first experimental evidence on the antiangiogenic potential of *C. mindanaense*, one of the most widely consumed species for spice and herbal medicine. This papers also establishes a scientific baseline for its pharmacological evaluation, and demonstrating the utility of the combined use of embryo morphometry and fractal-dimension analysis in screening for plant-derived angiogenesis modulators. Future research may consider increasing the sample size, inclusion of phytochemical and molecular characterization and use of a single image-based vascular endpoint which warrants further mechanistic and chemical investigations.

ACKNOWLEDGMENT

This study acknowledges the support of fellow researchers at the CNU RITBPB for the logistic support and the cinnamon farmers of San Antonio, Boljoon who kindly provided the researcher good quality bark of *C. mindanaense*.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

FUNDING

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

Abu-Reidah, I. M., & Taamalli, A. (2024). Promising phytoconstituents in antiangiogenesis drug development. *Nutraceuticals, 4*(4), 450–468. https://doi.org/10.3390/nutraceuticals4040027 MDPI

Alimpoos, M. R. G., Baguio, J. N., Bilbao, G. C. D., Cañada, L., Enerio, K., Jocom, N. M., et al. (2018). Antiangiogenic and morphological effects of *Cinnamomum cebuense* Kosterm. leaf extracts on *Anas platyrhynchos* embryonic development using an in vivo chorioallantoic membrane assay. *International Journal of Biosciences, 13*(1), 338–348. https://doi.org/10.12692/ijb/13.1.338-348

Almatroodi, S. A., Almatroudi, A., Alharbi, H. O. A., Khan, A. A., & Rahmani, A. H. (2024). Effects and mechanisms of luteolin, a plant-based flavonoid, in the prevention of cancers via modulation of inflammation and cell signaling molecules. *Molecules, 29*(5), 1093.

Bansode, R. R., Leung, T., Randolph, P., Williams, L. L., & Ahmedna, M. (2013). Cinnamon extract inhibits angiogenesis in zebrafish and human endothelial cells by suppressing VEGFR1, VEGFR2 and PKC-mediated MAP kinase. *Food Science & Nutrition, 1*(1), 74–82. https://doi.org/10.1002/fsn3.13

Carmeliet, P., & Jain, R. K. (2011). Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. *Nature Reviews Drug Discovery, 10*, 417–427. https://doi.org/10.1038/nrd3455 Nature

Datseris, G., Kottlarz, I., Braun, A. P., & Parlitz, U. (2023). Estimating fractal dimensions: A comparative review and open-source implementations. *Chaos: An Interdisciplinary Journal of Nonlinear Science, 33*(10). https://doi.org/10.1063/5.0160394 OpenAlex

Del Fierro, R. S., Maquilang, Q. M., Sanjorjo, R. A., Tradio, M. D., Shen, C. C., & Ragasa, C. Y. (2012). Secondary metabolites from *Cinnamomum cebuense*. *Journal of Medicinal Plants Research, 6*(11), 2146–2149. https://doi.org/10.5897/JMPR11.1379 ResearchGate

Deryugina, E. I., & Quigley, J. P. (2008). Chick embryo chorioallantoic membrane model systems to study and visualize human tumor cell metastasis. *Histochemistry and Cell Biology, 130*, 1119–1130.

Drake, C. J., & Little, C. D. (1995). Exogenous vascular endothelial growth factor induces malformed and hyperfused vessels during embryonic neovascularization. *Proceedings of the National Academy of Sciences of the United States of America, 92*(17), 7657–7661. https://doi.org/10.1073/pnas.92.17.7657 PubMed

Espineli, D. L., Agoo, E. M. G., Del Fierro, R. S., Shen, C. C., & Ragasa, C. Y. (2014). Cytotoxic and antimicrobial compounds from *Cinnamomum cebuense* Kosterm. *Pharmaceutical Chemistry Journal, 48*, 598–602.

Feneck, E., & Logan, M. (2020). The role of retinoic acid in establishing the early limb bud. *Biomolecules, 10*(2), 312. https://doi.org/10.3390/biom10020312

Folkman, J. (1971). Tumor angiogenesis: Therapeutic implications. *New England Journal of Medicine, 285*(21), 1182–1186. https://doi.org/10.1056/NEJM197111182852108 PubMed

Griffith, C. M., & Wiley, M. J. (1991). Effects of retinoic acid on chick tail bud development. *Teratology, 43*(3), 217–224. https://doi.org/10.1002/tera.1420430305 PubMed

Guidolin, D., Vacca, A., Nussdorfer, G. G., & Ribatti, D. (2004). A new image analysis method based on topological and fractal parameters to evaluate the angiostatic activity of docetaxel by using the Matrigel assay in vitro. *Microvascular Research, 67*(2), 117–124.

Hamburger, V., & Hamilton, H. L. (1951). A series of normal stages in the development of the chick embryo. *Journal of Morphology, 88*(1), 49–92. https://doi.org/10.1002/jmor.1050880104 Wiley Online Library

Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: The next generation. *Cell, 144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013 ScienceDirect

Hirata, O., Hirata, A., Murakami, Y., Shoji, M., Sakagami, H., & Fujisawa, S. (2005). Induction of cytotoxicity and apoptosis and inhibition of cyclooxygenase-2 gene expression by eugenol-related compounds. *Anticancer Research, 25*(5), 3263–3269.

Hoseinkhani, Z., Norooznezhad, F., & Mansouri, K. (2020). Medicinal plant extracts with antiangiogenic activity: Where do we stand? *Advanced Pharmaceutical Bulletin, 10*, 490–502.

Irvin, M. W., Zijlstra, A., Wikswo, J. P., & Pozzi, A. (2014). Techniques and assays for the study of angiogenesis. *Experimental Biology and Medicine, 239*(11), 1476–1488. https://doi.org/10.1177/1535370214529386 PubMed

Kostermans, A. J. G. H. (1986). *Cinnamomum* (Lauraceae), Part 1. *Ginkgoana, 6*, 30–68.

Lu, J., Zhang, K., Nam, S., Anderson, R. A., Jove, R., & Wen, W. (2010). Novel angiogenesis inhibitory activity in cinnamon extract blocks VEGFR2 kinase and downstream signaling. *Carcinogenesis, 31*(3), 481–488. https://doi.org/10.1093/carcin/bgp292 PubMed

Lugano, R., Ramachandran, M., & Dimberg, A. (2020). Tumor angiogenesis: Causes, consequences, challenges and opportunities. *Cellular and Molecular Life Sciences, 77*(9), 1745–1770. https://doi.org/10.1007/s00018-019-03351-7 Springer

Mada, J., & Tokihiro, T. (2022). Pattern formation of vascular network in a mathematical model of angiogenesis. *Japan Journal of Industrial and Applied Mathematics, 39*(1), 351–384.

Maden, M. (2002). Retinoid signalling in the development of the central nervous system. *Nature Reviews Neuroscience, 3*, 843–853.

Manikandan, P., Murugan, R. S., Priyadarsini, R. V., Vinothini, G., & Nagini, S. (2010). Eugenol induces apoptosis and inhibits invasion and angiogenesis in a rat model of gastric carcinogenesis induced by MNNG. *Life Sciences, 86*(25–26), 936–941.

Mapanao, A. K., Che, P. P., Sarogni, P., Sminia, P., & Giovannetti, E. (2021). Tumor-grafted chick chorioallantoic membrane as an alternative model for biological cancer research and conventional/nanomaterial-based theranostics evaluation. *Expert Opinion on Drug Metabolism & Toxicology, 17*, 947–968.

Nitzsche, B., Rong, W. W., Goede, A., et al. (2022). Coalescent angiogenesis—Evidence for a novel concept of vascular network maturation. *Angiogenesis, 25*, 35–45.

Parsons-Wingerter, P., Lwai, B., Yang, M. C., Elliott, K. E., Milaninia, A., Redlitz, A., Clark, J. I., & Sage, E. H. (1998). A novel assay of angiogenesis in the quail chorioallantoic membrane: Stimulation by bFGF and inhibition by angiostatin according to fractal dimension and grid intersection. *Microvascular Research, 55*(3), 201–214.

Ragasa, C. Y., Espineli, D. L., Agoo, E. M. G., & Del Fierro, R. S. (2013). Chemical constituents of *Cinnamomum cebuense*. *Chinese Journal of Natural Medicines, 11*(3), 264–268. https://doi.org/10.1016/S1875-5364(13)60026-6 ScienceDirect

Rao, P. V., & Gan, S. H. (2014). Cinnamon: A multifaceted medicinal plant. *Evidence-Based Complementary and Alternative Medicine, 2014*, 642942.

Rhinn, M., & Dollé, P. (2012). Retinoic acid signalling during development. *Development, 139*, 843–858.

Ribatti, D. (2017). The chick embryo chorioallantoic membrane (CAM) assay. *Reproductive Toxicology, 70*, 97–101.

Ribatti, D. (2022). The chick embryo chorioallantoic membrane (CAM) assay in the study of angiogenesis. *Biomolecules, 12*, 1297.

Schäfer, M., Ettner-Sitter, A., Brand, L., Lotter, S., Vakilipoor, F., Aung, T., Haerteis, S., & Schober, R. (2024). The chorioallantoic membrane model: A 3D in vivo testbed for design and analysis of MC systems. In *Proceedings of the 11th Annual ACM International Conference on Nanoscale Computing and Communication* (pp. 47–53).

Stepanova, D., Byrne, H. M., Maini, P. K., & Alarcón, T. (2024). Computational modeling of angiogenesis: The importance of cell rearrangements during vascular growth. *WIREs Mechanisms of Disease, 16*(2), e1634.

sStratford, T., Horton, C., & Maden, M. (1996). Retinoic acid is required for the initiation of outgrowth in the chick limb bud. *Current Biology, 6*(9), 1124–1133. https://doi.org/10.1016/S0960-9822(02)70679-9

Thanekar, D., Dhodi, J., Gawali, N., Raju, A., Deshpande, P., Degani, M., & Juvekar, A. (2016). Evaluation of antitumor and anti-angiogenic activity of bioactive compounds from *Cinnamomum tamala*: *In vitro, in vivo* and *in silico* approach. *South African Journal of Botany, 104*, 6–14. https://doi.org/10.1016/j.sajb.2015.09.014