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Original Article

The Antiangiogenic Activity of the *Pereskia grandifolia* (Seven Star Needle) Crude Leaf Extract Using the Chick Embryo Chorioallantoic Membrane (CAM) Assay

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Abstract

Background: *The study focused on the anticancer potential of Pereskia grandifolia, specifically its antiangiogenic properties.*

Methods: *Soxhlet extraction and rotary evaporation yielded a crude leaf extract containing flavonoids, phenols, tannins, steroids, and alkaloids. An ex-ovo Chorioallantoic Membrane (CAM) Assay was performed on twenty-one 4-day-old chicken embryos, divided into three groups: the experimental group (40.5 mg/50 mcL crude extract), positive control (7.5 mcg/50 mcL dexamethasone), and negative control (50 mcL distilled water).*

Results: *The experimental group exhibited the highest inhibition of blood vessel length (43.99%), significantly greater than the positive control group (28.51%) and negative control group (0%). Statistical analysis confirmed significant differences in blood vessel length inhibition ($t = 2.590$, $p = 0.024$), number ($F = 93.415$, $p < 0.001$, $w^2 = 0.80$), and vessel density ($F = 94.343$, $p < 0.001$).*

Conclusion: *These findings suggest Pereskia grandifolia's potential as an antiangiogenic agent for cancer therapy.*

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Keywords

Pereskia grandifolia, antiangiogenic activity, chorioallantoic membrane (CAM) assay, drug therapy, dexamethasone, flavonoids

INTRODUCTION

The World Health Organization [WHO] (2022) classifies Cancer as one of the leading causes of mortality, with over 10 million deaths all over the world in 2020 alone. It is a disease characterized by the disruption of the normal cell division cycle of the body, leading to unusual and uncontrollable growth of cells. When cells grow without necessity, new lumps of tissue form and are referred to as tumors. Similar to the ideal development of the body's normal cells, tumors require oxygen and nutrients to proliferate. Pathological tumor angiogenesis is characterized by a distorted vasculature structure, which advances to enhanced permeability and retention effect, which may lead to cancer cells' intravasation and heightened metastasis.

Many Filipino families, including those with higher incomes, face financial strain due to costly cancer treatment and limited public program coverage (Ngelangel et al., 2018). Beyond financial concerns, cancer therapy causes severe side effects that affect patients' quality of life (Smith et al., 2019). Doctors recommend antineoplastic drugs based on cancer type, stage, and treatment risks. Angiogenesis inhibitors, a unique class of anticancer agents, block blood vessel growth supporting tumors rather than targeting tumor cells directly (National Cancer Institute, 2018). The FDA has approved several of these inhibitors, mainly targeting VEGF or its receptor.

Recently, vascular normalization has become a significant area of interest as a complementary approach to normalizing tumor vasculature (López-Camarillo et al., 2020). Like many other diseases, the emphasis on prevention outweighs the importance of finding a cure. Acknowledging the potential presence of active pharmaceutical constituents within plant materials, an investigative approach into the antiangiogenic properties of lesser-known botanical extracts, like that of *Pereskia grandifolia*, holds promise in uncovering novel avenues for cancer treatment.

Pereskia grandifolia, or Seven Star Needle, is a shrub-like cactus that thrives in warm climates and requires regular watering when in leaf (Zareisedehizadeh et al., 2014). Unlike most cacti, it can be easily propagated from fresh stem cuttings or seeds without a healing period (University of Wisconsin, 2019). Current classification divides the subfamily *Pereskioideae* into two genera: *Pereskia* (17 species) and *Maihuenia* (2 species) (Hunt, 2016, as cited in Maciel et al., 2019). *Pereskia grandifolia* is less studied than its relative, *Pereskia bleo*, which is known for its anti-cancer, anti-tumor, anti-inflammatory, and anti-rheumatic properties (Siska et al., 2023). Given its use in treating hypertension and diabetes, *Pereskia grandifolia* may have similar benefits. While some studies have explored its properties, research on its antiangiogenic effects remains limited (National University of Singapore, 2019). A study by Johari & Khong (2019) found that the *Pereskia* genus contains carotenoids, alkaloids, flavonoids, lactones, sterols, terpenoids, fatty acids, phytosterol glycosides, and phenolic compounds, which attributed to its antioxidant, anticancer, antinociceptive, and antibacterial property. Given these findings, verifying whether *Pereskia grandifolia* also possesses anticancer and antiangiogenic properties would be highly beneficial.

The study aims to investigate the antiangiogenic potential of the crude leaf extract from *Pereskia grandifolia*, a member of the *Pereskia* genus, in preventing angiogenesis and further proliferation of tumor formations with the use of the Chorioallantoic Membrane (CAM) Assay using chicken eggs. The CAM, a highly vascularized extraembryonic membrane, provides an ideal environment for human tumor cell proliferation. Pinto et al. (2020) describe the CAM Assay as a low-cost, reproducible, and immune-incompetent model for studying tumor development. It allows real-time visualization of angiogenesis and direct application of test substances.

To assess the ex-ovo antiangiogenic activity of a plant extract, a positive control group is essential for comparison. Synthetic glucocorticoids, like dexamethasone, are commonly used in cancer treatments for their angiostatic effects (Liu & Goodwin, 2020). Uslu et al. (2022) found that a 0.1 mg/kg dose of dexamethasone reduced neural tube thickness and mitosis in chick embryos. As a corticosteroid, dexamethasone suppresses inflammation, inhibits VEGF expression, induces endothelial cell apoptosis, and prevents vascular proliferation (Liu et al., 2019).

The study includes three treatment groups: Positive Control, Negative Control, and Experimental Group. This design follows Bactin et al. (2022), who used retinoic acid as the positive control, carrot peel extract as the experimental treatment, and distilled water as the negative control in a CAM model. These groups provide a sufficient basis for comparing and validating the study's outcomes.

METHODS

The study was a true experimental design utilizing a pretest-posttest control group study, which involved pre-intervention and post-intervention. It measured how an intervention or treatment affects an outcome variable by comparing the scores of the same research subjects before and after the treatment exposure. A randomized controlled trial was used to determine the antiangiogenic properties of a crude leaf extract derived from *Pereskia grandifolia* (Seven Star Needle) through the context of the chorioallantoic membrane (CAM) of four-day-old, fertilized chicken eggs and incubated for 48 hours after treatment.

Collection of the Plant Sample

The researchers procured *Pereskia grandifolia* leaf samples at Barangay Tunghaan, Minglanilla, Cebu. Fresh, healthy, and mature leaves of *Pereskia bleo* were harvested and utilized for methanolic extraction (Tan et al., 2005). Nichols-Orians (1992), mature leaves are described as fully expanded and dark green in color. In addition, the study of de Souza et al. (2022) found that the production of bioactive compounds as well as the antioxidant activity of the *Pereskia* aculeata Mill. remained the same regardless of the season it was harvested in.

Preparation and Extraction of the Crude Leaf Extract

In a study by Manaf et al. (2014), utilizing circulating air oven at 45°C for 5 hours, drying 30 g of fresh leaves, and subjecting the dried plant sample to 180 mL of 60% methanol yields the highest phenolic content with antiangiogenic activity. Following the drying process, the dried plant samples were finely pulverized using a blender, all of which were performed in Room 423 of Cebu Doctors' University. In this study, the researchers utilized more than eleven-fold of the plant sample and solvent, which was 1,998 mL of 60% methanol for 333 g of fresh plant sample or 47.85 g after pulverization.

The researchers performed the Soxhlet extraction of the *Pereskia grandifolia* extract in Room 421. The pulverized plant sample was placed inside a tea bag and then placed in a thimble. Subsequently, methanolic solvent was placed in a round-bottom flask attached to an isomantle, along with a Soxhlet extractor and condenser. After heating with the isomantle, the solvent evaporated and moved through the apparatus into the condenser. After the Soxhlet extraction, the plant extract was then transferred to another container, and it underwent further evaporation process using the rotary evaporator at 40°C (Kintek Solution Ltd, n.d.), with speed level 5 (95-100 rpm) of the Stuart RE300 rotavap, conducted within the CDU Experimental Research Laboratory. The *Pereskia grandifolia* crude leaf extract was weighed and found to be 8.8 g after rotavap. A flame test was conducted to ensure that the methanol was removed entirely from the crude plant extract, and confirmatory tests of the plant's hypothesized phytochemicals present were conducted at the Pharmaceutical Chemistry Laboratory, Room 424.

The crude plant extract featured a distinct and visually striking green color, with a runny yet heterogeneous consistency, and a characteristic grassy odor. The percentage of the actual yield produced based on the dried plant sample and the crude extract was calculated using the formula utilized by Bhandari et al. (2015),, was found to be 18.39%.

Preparation of Positive Control

The study utilized one vial of Dexamethasone 5 mg/mL as a positive control drug. The eggs utilized weigh an average of 75 g. Therefore, an aliquot of 0.3 mL of the Dexamethasone solution was diluted in a 9.7 mL of 0.9% sodium chloride solution for injection to obtain 10 mL of 7.5 mcg/0.05 mL or 7.5 mcg/50 mcL of Dexamethasone concentration, which was administered on each fertilized egg of the positive control group.

Preparation and Randomization of Research Subjects

The study utilized chicken eggs from a specific poultry farm, "Ilaya Livestock Agri-Farm," located in Purok Sambag 1, Ilaya, San Fernando, Cebu. Each group, namely the experimental group, positive control group, and negative control group, was randomly assigned seven (7) eggs per group. The eggs were then incubated for 4 days to allow the CAM to develop. Then, for 72 hours, the four-day-old eggs were incubated in an incubator that automatically tilts every 2 hours and equipped with a temperature and humidity monitor. They were arranged laying down vertically and separated based on their group assignment. Within the incubator, the eggs were tilted continuously for 2 hours, with the humidity maintained at 60-62% at 37.5-38.2°C (Dohle et al., 2009).

On the seventh day, the test subjects were removed from the incubator and were subjected to candling to identify viability. Then, each egg was positioned horizontally with the pencil mark on top and cracked on the edge of a triangular prism, perpendicular to the egg's long axis, and then transferred into a clean, 100 mm by 15 mm petri dish. The eggs in the petri dish underwent further viability assessment based on observed heartbeat and vasculature. They were positioned to allow clear visibility of the blood vessels atop an intact

yolk. The eggs with visible heartbeat and prominent vascularization were deemed viable and considered for inclusion in the research.

Filter paper discs were prepared, which served as a marker for treatment induction. They were placed in a sterilization pouch to be autoclaved to ensure sterility and were used as appropriate carriers for the treatment to be administered. The paper discs were then carefully placed in each egg culture group. At this time, no interventions have been introduced.

The test subjects were inducted with 50 mcL of a 7.45-pH phosphate-buffer solution on the paper discs to maintain the egg's stability throughout the experiment and prevent the cells from rupturing or dehydrating (Mohan, 2006). The research subjects were then promptly returned to the incubator to continue incubating for another 24 hours.

After the fertilized chicken eggs were incubated for 24 hours, the Petri dishes containing the eggs were removed from the incubator. Prior to treatment, the vascularization of the egg cultures was measured using a dissecting stereoscope to identify the baseline data.

Induction of Treatment

Each group was applied with sterilized paper discs and then administered again with phosphate buffer prior to the induction of their allocated treatment using a micropipette: 40.5 mg/50 mcL per egg for the experimental group, 50 mcL for the negative control group, and 7.5 mcg/50 mcL for the positive control group. The egg cultures were placed again in the incubator for another 48 hours after their respective experimental and control treatments were applied.

Collection of Data

After 48 hours, on the tenth day of the procedure, the egg cultures were removed from the incubator to initiate the examination of the vascularization observed. The phosphate buffer was then re-administered. Finally, the CAM was placed under a stereoscope for image acquisition. The researchers utilized the ImageJ Angiogenesis Analyzer Software to determine the inhibition of angiogenesis progression, providing a semi-automated assessment of angiogenesis and measuring parameters such as vessel formation, vessel length, and vessel density. It is specifically designed to quantify angiogenesis tubules.

Determination of Antiangiogenic Activity

The angiogenesis activity can be expressed as the percent angiogenesis inhibition of vessel length and calculated using the same formula utilized by Umar et al. (2014):

$$\text{Percent Inhibition} = \left[1 - \left(\frac{A_0}{A}\right)\right] \times 100$$

Where A_0 is the average length of blood vessels in mm of the experimental group or positive control group, and A is the average length of the negative control group.

The results indicate a positive percent inhibition in the experimental and positive control groups, implying that the *Pereskia grandifolia* crude leaf extract and dexamethasone exhibit anti-angiogenic activity. Additionally, the determination of the significant difference between the percent inhibition of experimental and positive control groups infer that the treatment groups are similar or varied in terms of the extent of blood vessel length inhibition.

RESULTS

Results of Phytochemical Screening

The study included phytochemical screening consisting of confirmatory tests for the hypothesized phytochemicals of the crude plant extract, following methods from Kancherla et al. (2019). The presence of flavonoids was confirmed using the alkaline reagent test, to which the solution gradually turned colorless, indicating a positive result. The Bromine water test confirmed the presence of phenols, with the disappearance of bromine's color upon dropwise addition indicating a positive result. The presence of tannins was confirmed

using the Ferric Chloride Test, which produced a green precipitate, indicating a positive result. The presence of steroids was confirmed using the Salkowski Test, which produced a red color, indicating a positive result. The presence of alkaloids was confirmed using Dragendorff's Test, with the formation of an orange-red precipitate indicating a positive result. The crude extract was also tested for the absence of methanol using a flame test, following the procedure from the study of Rohrig (2015). When exposed to an open flame, the extract did not ignite or alter the flame color, confirming that no methanol was present.

All of the phytochemicals tested had positive results. However, flavonoids have benefits that can potentially contribute to the antiangiogenic activity of *Pereskia grandifolia* leaves. According to Hassan et al. (2014), Flavonoids exercise their anticancer effects by altering key pathways in cancer etiology. These potent antiangiogenic and antioxidant substances inhibit the metabolic activation of carcinogens in the early stages of cancer development. During the advancement stages, flavonoids induce apoptosis, inhibit angiogenesis, prevent the proliferation of cancer cells, and halt tumor spread.

The Mean Blood Vessel Number, Density, and Length of the Chicken Embryos Between the Experimental and Control Groups on Chicken Embryos Formed After Post-Intervention

Blood vessel number refers to the number of blood vessel branches or the number of blood vessels formed at a certain duration. Blood vessel density is the proportion or quantity of blood vessels within a specific area of tissue, quantifying the number of blood vessels present per unit of tissue space. The blood vessel length per egg was determined using the ImageJ angiogenesis analyzer. After the induction of treatment, the samples were incubated for 48 hours.

Table 1. *Mean Blood Vessel Number, Density, and Length of the Chicken Embryos Between the Experimental and Control Groups on Chicken Embryos Formed After Post-Intervention (n = 21)*

Treatment Group	Blood Vessel Number Mean (SD)	Blood Vessel Density (mm^2) Mean (SD)	Blood Vessel Length (mm) Mean (SD)
Experimental Group: 40.5 mg/50 mcL Crude Leaf Extract (n = 7)	30.29 (10.66)	3.76 (0.56)	40.80 (12.77)
Positive Group: 7.5 mcg/50 mcL Dexamethasone (n = 7)	44.29 (2.06)	4.34 (0.30)	52.07 (5.33)
Negative Group: 50 mcL Distilled Water (n = 7)	62.00 (2.89)	6.67 (0.27)	72.84 (9.23)

The Percentage of the Blood Vessel Length Inhibited in the Experimental Group, Positive Group, and Negative Group

Percent inhibition pertains to the percent of blood vessel length inhibited after exposure to treatment by comparing the results of the experimental and control groups. If there is a comparable inhibitory percentage between the experimental group and the positive control group, the sample may be deemed to have an antiangiogenic activity. This was determined by measuring the average blood vessel length using the ImageJ Angiogenesis Analyzer Software. The formula by Umar et al. (2014) was used to calculate the percent inhibition. The data obtained from the experimental and/or positive group was further divided by the data obtained from the negative control group and multiplied by 100.

Table 2. *Percent Inhibition of the Blood Vessel Length of the Chicken Embryos*

Treatment Group	n	Percent Inhibition (%)
Experimental Group: 40.5 mg/50 mcL Crude Leaf Extract	7	43.99
Positive Group: 7.5 mcg/50 mcL Dexamethasone	7	28.51
Negative Group: 50 mcL Distilled Water (n = 7)	7	0

Significant Difference between the Experimental and Control Groups as to Percent Inhibition of Blood Vessel Length

An independent samples t-Test for the percent inhibition of blood vessel length was utilized to determine the degree of significance of the variables in comparison to each other. The table below is based on the values of the percent inhibition of the experimental and control groups.

Table 3. *Independent Samples t-Test for the Percent Inhibition of Blood Vessel Length between the Experimental and Positive Control Group on Chick Embryo Eggs using the CAM Assay*

Treatment Group	Mean Difference	*t* value	df	*p*-value	Conclusion
Experimental Group: 40.5 mg/50 mcL Crude Leaf Extract Positive Group: 7.5 mcg/50 mcL Dexamethasone	16.567	2.590	12	.024	Significant

Significant Difference between the Experimental and Control Groups as to Mean Number of Blood Vessels

Welch Analysis of Variance (ANOVA) was utilized to determine the significant difference between the experimental and control groups in terms of mean blood vessel number. Determining the significant difference among the three groups implies that the treatment affected blood vessel development.

Table 4.1 *Welch ANOVA on the Blood Vessel Number between the Experimental Group and Control Groups on Chick Embryo Eggs using the CAM Assay*

	F	df1	df2	*p*-value	Effect Size (w^2)	Conclusion
Welch	93.415	2	10.476	.000	.80	Significant

The Games-Howell Post-Hoc Test was also conducted to identify the particular treatment group pairs that differ significantly from one another. This assumes that the compared groups have unequal variances. The results are presented in the table below.

Table 4.2 *Games-Howell Test on the Pairwise Difference of Blood Vessel Number between the Experimental Group and Control Group*

Group Pair	Mean Difference	*p*-value	Conclusion
7.5 mcg/50 mcL Dexamethasone (Positive Group) vs. 40.5 mg/50 mcL Crude Leaf Extract (Experimental Group)	14.00	.030	Significant
50 mcL Distilled Water (Negative Group) vs. 40.5 mg/50 mcL Crude Leaf Extract (Experimental Group)	31.71	.000	Significant
50 mcL Distilled Water (Negative Group) vs. 7.5 mcg/50 mcL Dexamethasone (Positive Group)	17.71	.000	Significant

Significant Difference between the Experimental and Control Groups as to Mean Density of Blood Vessels

The one-way ANCOVA (analysis of covariance) was used to determine whether there were significant differences between the experimental and control groups in terms of the mean density of blood vessel formation at a 5% level of significance.

Table 5. *One-Way ANCOVA on the Blood Vessel Density between the Experimental Group and Control Groups on Chick Embryo Eggs using the CAM Assay*

Source	Sum of Squares	*df*	Mean Square	*F* value	*p*-value	Partial Eta Squared	Conclusion
Pre	.184	1	.184	1.175	.294	.065	Not Significant
Group	29.474	2	14.737	94.343	.000	.917	Significant
Error	2.656	17	.156				
Total	544.950	21					

DISCUSSION

Mean Blood Vessel Number, Density, and Length of the Chicken Embryos Between the Experimental and Control Groups on Chicken Embryos Formed After Post-Intervention

According to the parameters outlined in this study, a lower blood vessel count signifies potential antiangiogenic activity. The eggs treated with 40.5 mg/50 mcL crude leaf extract showed the lowest blood vessel number mean of 30.29±10.66, followed by the eggs treated with 7.5 mcg/50 mcL dexamethasone showed an average of 44.29±2.06, and lastly, the eggs treated with 50 mcL of distilled water showed the highest average of 62.00±2.89. Furthermore, the variance in the mean blood vessel count reduction between the experimental and positive control groups can be attributed to their differing concentrations: 40.5 mg/50 mcL and 7.5 mcg/50 mcL, respectively. Conversely, since the negative control group recorded the highest blood vessel number among the three groups, it can be inferred that it did not display antiangiogenic activity.

The eggs treated with distilled water exhibited the greatest blood vessel density of all the treatment groups, with a mean of 6.67 and a standard deviation of 0.27. Following the negative control, dexamethasone-treated eggs had a mean of 4.34 and a standard deviation of 0.30. Out of all the treatment groups, the eggs treated with the crude leaf extract exhibited the lowest mean blood vessel density, at 3.76, and a standard deviation of 0.56. The results indicate that the negative group, characterized by the highest blood vessel density value, exhibited no antiangiogenic activity, unlike the experimental group, which had the lowest blood vessel density, followed by the positive group.

The lowest mean value was 40.80 mm, as exhibited by the experimental group. By having the lowest value calculated, the experimental treatment showed the greatest reduction in blood vessel length. In the same manner, the positive control group exhibited a substantial reduction in blood vessel length based on its blood vessel length mean of 57.07 mm. The negative control group, having a blood vessel length mean of 72.84 mm, implies minimal reduction in blood vessel length. The post-intervention data of the blood vessel length are utilized to identify the percentage of inhibition for anti-angiogenic properties of the experimental, positive control, and negative control groups.

The Percentage of the Blood Vessel Length Inhibited in the Experimental Group, Positive Group, and Negative Group

It is evident that after the induction of treatment, the experimental group had the highest inhibition percentage of 43.99%, followed by the positive group, which had 28.51%. With these findings, it can be inferred that based on the parameter of percent inhibition of blood vessel length, the experimental treatment of the pure *Pereskia grandifolia* crude leaf extract exhibits antiangiogenic activity. Other parameters beyond this measurement are presented and discussed in the succeeding results to corroborate the antiangiogenic activity of the *Pereskia grandifolia* crude leaf extract. Moreover, while the experimental group exhibited a higher percentage of blood vessel length inhibition than the positive group, noteworthy inhibition of blood vessel length was still observed in the positive group, indicating potential antiangiogenic activity at 7.5 mcg/50 mcL dexamethasone dose. Conversely, the negative group exhibited 0% inhibition, indicating no angiogenesis inhibitory activity.

Significant Difference between the Experimental and Control Groups as to Percent Inhibition of Blood Vessel Length

A significant difference was established in the percent inhibition of blood vessel length between the experimental group and the positive group using the independent samples t-test. Based on the findings, there is a significant difference between the two groups, implying that the experimental intervention exhibits a relatively higher inhibition than the positive control group. Additionally, the p-value of 0.024 is less than the typical significance level of 0.05, indicating that the observed difference in percent inhibition is statistically significant. In addition, it was determined that the effect size was small based on the values of $t(12) = 2.590$, $p = .024$, with an eta squared statistic of 0.36. Despite the small effect size indicating a significant difference between the positive and experimental groups, the experimental group has a different level of inhibition than the positive group. Since the p-value is below 0.05, we reject the null hypothesis, which states no significant

difference in percent inhibition between the experimental and control groups. Thus, the result suggests that the experimental treatment's effectiveness exhibits better inhibition of blood vessel length compared to the positive control group at their respective doses.

Significant Difference between the Experimental and Control Groups as to Mean Number of Blood Vessels

The results of the analysis of variance (ANOVA) are shown as $F(2,10.476) = 93.415$, $p < .05$. With the p-value indicating the degree of statistical significance and the degrees of freedom represented by $F(2,10.476)$, this indicates that there was indeed a significant difference among the groups. This suggests that the treatment type administered on each egg was crucial in influencing blood vessel development in the chick embryos. This idea is further supported by the effect size, shown as 80%, representing the proportion of the variation in blood vessel numbers attributed to the type of treatment applied to the eggs. In this case, it implies that the treatments applied can account for a substantial amount (80%) of the variability in blood vessel formation.

The significant difference observed between the positive group and the experimental group ($p = 0.030$) suggests varying levels of effectiveness in inhibiting blood vessel number. Specifically, the experimental group's mean difference of 14.00 compared to the positive group implies a notable difference in their impacts on angiogenesis. Comparing the experimental group to the negative group yields even more pronounced differences ($p = 0.000$) in blood vessel number, with a mean difference of 31.71. This highlights the potential efficacy of the experimental group in inhibiting blood vessel formation compared to the negative group. Furthermore, comparing the positive group to the negative group shows a substantial difference ($p = 0.000$) in blood vessel number, with a mean difference of 17.71. This indicates that the positive group has a significant impact on blood vessel formation compared to the negative group as well. The significant differences observed suggest varying degrees of effectiveness in inhibiting blood vessel formation, with the experimental group demonstrating promising results compared to both the positive and negative control groups. These findings imply that the positive control and experimental treatments under investigation have antiangiogenic activity, with the experimental treatment showing prospective therapeutic potential in cancer treatment.

Significant Difference between the Experimental and Control Groups as to Mean Density of Blood Vessels

There is no significant difference among the three groups during pretreatment in terms of blood vessel density. This indicates that before the experimental and control treatments were administered, the blood vessel density of each group was comparable to one another. Thus, the baseline blood vessel density measurements for all three groups can be considered reasonably close to one another. This implies that the groups under comparison were initially on an equal basis, which can be important during the CAM Assay because it helps guarantee that any observed variations in the outcomes are more likely to result from the intervention or treatment under study rather than pre-existing disparities.

CONCLUSION

In conclusion, the results suggest that the *Pereskia grandifolia* (Seven Star Needle) crude leaf extract exhibited antiangiogenic activity as demonstrated by the Chorioallantoic Membrane (CAM) Assay on fertilized chicken eggs. Significant difference was observed across the pure *Pereskia grandifolia* crude leaf extract-treated group and the control groups in terms of the percentage inhibition of blood vessel length, average number of blood vessels, and blood vessel density.

Recommendations

Future studies should standardize concentration determination for reliable comparisons with existing treatments to optimize the therapeutic use of *Pereskia grandifolia* crude leaf extract. Research efforts should also focus on isolating and characterizing bioactive compounds, mainly phenols and flavonoids, to enhance understanding of their antiangiogenic properties and therapeutic potential.

Author Contributions

Abala: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft; **Acuña:** Funding acquisition, Investigation, Methodology, Resources, Visualization & Presentation, Writing – original draft & editing; **Daan:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Visualization, Writing – original draft; **Dico:** Funding acquisition, Investigation, Methodology, Resources, Writing – original draft; **Fontillas:** Data curation, Funding acquisition, Investigation, Methodology, Software, Resources, Writing – original draft; **Dela Cruz:** Formal analysis, Supervision, Validation, Writing – review & editing

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Ethical Approval

The study protocol was reviewed and exempted by the Cebu Doctors' University – Institutional Animal Care and Use Committee (CDU-IACUC) under IACUC Code: EX2024-02-Abala-CAMAssay on January 24, 2024. It was subsequently referred to the Institutional Biosafety Committee (IBC), which granted approval for implementation under Protocol No. BSPh_2024_01 on February 20, 2024. The ethical clearance remains valid until February 20, 2027.

Competing interest

The authors declare no conflicts of interest.

Data Availability

Data will be made available by the corresponding author on request.

Declaration of Artificial Intelligence Use

In this work, the authors did not utilize artificial intelligence (AI) tools and methodologies.

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