Anti-Angiogenic Effect of Saccostrea Cucullata (Sisi) In Varying Doses to Anas Platyrhynchos Domesticus (Itik)

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Abstract

This study examines the Saccostrea cucullata (Sisi) extract's anti-angiogenic effectiveness with the use of varying doses administered into Anas platyrhynchos domesticus (Itik) using Chorioallantoic Membrane (CAM) Assay. The study used an experimental design to assess the extract's efficacy compared to a negative control group and to determine the minimum dose required for observable effect, such various doses are: 20 mg/mL, 30 mg/mL, and 50 mg/mL. Saccostrea cucullata (Sisi) extract showed significant anti-angiogenic activity, with the strongest inhibition of blood vessel growth observed at the highest dose (50 mg/mL). It implies that Saccostrea cucullata (Sisi) extract could be used therapeutically to prevent the formation of new blood vessels in diseases such as cancer, which cause the development of abnormal blood vessels.

Introduction:-

Angiogenesis is crucial for the provision of nutrients and oxygen during the development of cancer. The Tumor growth promotes the production of angiogenic factors, such as Fibrinogen Growth Factor (FGF), Vascular Endothelial Growth Factor (VEGF), and Angiopoietin, which activate integrins. Angiogenic factors such as FGF, VEGF, and Angiopoietin enhance permeability, migration, cell proliferation, and tube formation (Li et al., 2017). Since tumor cells need a blood supply to survive and metastasize, inhibiting the process of Angiogenesis is essential to hinder cancer progression. In light of this, antiangiogenesis refers to suppressing blood vessel growth to disrupt the blood supply to tumors (Cleveland Clinic, 2022). According to a study, antiangiogenic processes have been successfully achieved through drug medications in cancer therapies, highlighting the practical application of angiogenesis inhibition in clinical settings (Lopes-Coelho et al., 2021). In the broader context of the anti-angiogenesis field, oysters have gained attention, mainly those commonly farmed in saltwater environments. As oysters are known for their rough, irregular shells,they are among the most widely farmed saltwater bivalves globally. According to the Industry Strategic Science and Technology (n.d) report, oysters are valuable for providing income to shellfish farmers for their health benefits, primarily due to their high zinc content (Li et al., 2019). The soft tissues of oysters contain bioactive compounds, metallic properties, and enzymes. Some bioactive compounds such as antioxidants and anticancer agents, have demonstrated the ability to inhibit Angiogenesis (Maravich, 2023). Therefore, oysters are crucial to global ocean health due to their significant nutritional and therapeutic benefits, making them widely consumed and studied.

Among the various oyster species, Saccostrea cucullata holds particular significance. This oyster species, called the rock oyster or "Sisi," is of substantial economic value in several tropical countries (Thanormjit et al., 2020). The cultivation of oysters at Bacoor Bay in Cavite, Philippines, has a long history (Asia Farming, 2023). The Philippines has four distinct species of oysters cultivated, with Saccostrea cucullata being one of the most prominent. This specific specie of oyster is endemic to Negros Occidental, Pangasinan, and Cavite; Saccostrea cucullata is an elongated shell with a flat or slightly convex correct valve and a smaller left valve attached to substrates (Amaral et al., 2020). The soft tissues of this species contain a high concentration of zinc, as well as copper (Cu), manganese (Mn), and iron (Fe), which contribute to its significant nutritional value and economic potential (Rodrigues et al., 2021). This nutritional composition highlights its potential for both health-related applications and as a commercial resource. Given the demonstrated potential of Saccostrea cucullata, this study aims to study its antiangiogenic

properties. Specifically, it seeks to determine the minimum dose required for the antiangiogenic effect of Saccostrea cucullata (Sisi) ethanolic extract when administered to Anas platyrhynchos domesticus (Itik) using the Chorioallantoic Membrane (CAM) Assay. The study will focus on observing changes associated with the inhibition of Angiogenesis, contributing to the growing research on natural compounds with medical benefits

Conceptual/Theoretical Framework

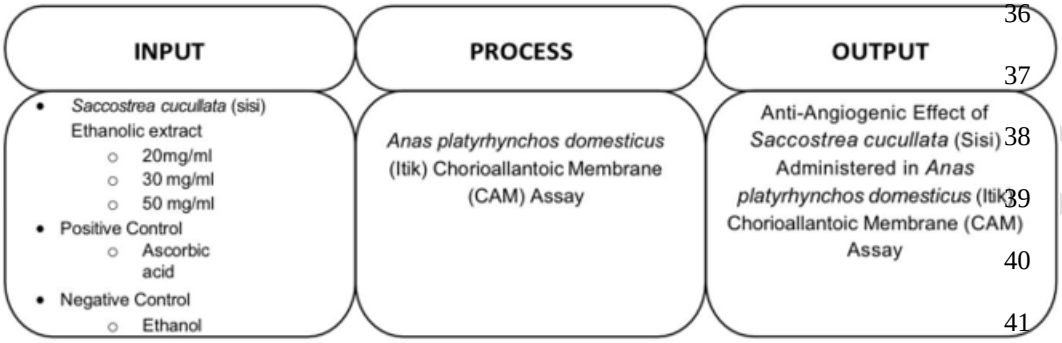


Figure 1

The framework uses Saccostrea cucullata (Sisi) extract via the Ethanolic extraction method—the input administered in Duck Anas platyrhynchos domesticus (Itik) Chorioallantoic Membrane (CAM) Assay. The purpose of this study is to observe the antiangiogenic effect of Saccostrea cucullata (Sisi) extract when administered in Anas platyrhynchos domesticus (Itik) Chorioallantoic Membrane (CAM) Assay.

Research Design

The experimental design involved the administration of extract on Anas platyrhynchos domesticus (Itik) Chorioallantoic Membrane (CAM) Assay to evaluate the effectiveness of the Antiangiogenic effect of Saccostrea cucullata (Sisi) extract at different doses.

Specimen Collection

Collection, Transportation, and Storage of Saccostrea cucullata (Sisi)

The researchers collected Saccostrea cucullata (Sisi/Rock oysters) in Sta. Elena, Hagonoy Bulacan, Philippines. During the collection,the oysters were transported and placed with the cupped half of the shell facing downward, kept moist by covering the shell using a damp towel to protect against mechanical damage (Food and Agriculture Organization, n.d). The oysters were then exposed to natural air (27-30°C) to mimic the actual transport conditions (Nuñal et al., 2023). As for the storage,the oysters were placed in a large plastic container and stored at room temperature. The temperature and storage time of 1 to 12 days showed no significant effect on the quality and survival of oysters (Da Costa, 2018). The healthy live oysters were identified by weight, solid sound when tapped, and tightly closed when handled (Food and Agriculture Organization, n.d). The oysters with open or cracked shells are discarded as they are already dead or contaminated.

Collection and Transportation of Anas platyrhynchos domesticus ("Itik") Eggs

For the experimental Chorioallantoic Membrane (CAM) Assay,the fertilized duck eggs were collected at a local distributor in Pateros, with an estimated age of 8 days. The duck eggs were then transported in a container cushioned in wheat husks to maintain the proper temperature and humidity for transportation (Tabamo, 2021). Upon arrival,the researchers used seventy percent (70%) ethanol to clean the egg's shell by wiping its surface with cotton to prevent debris contamination once the embryo opened (Gamallo, 2016). After cleansing the shell, duck eggs were already set for experimentation.

Specimen Identification of Saccostrea cucullata (Sisi)

To authenticate oyster species, the researchers submitted the samples to the Marine Biologist at the Bureau of Fisheries and Aquatic Resource, Quezon City

Specimen Identification of Anas platyrhynchos domesticus (Itik) Eggs

To authenticate the duck egg species, Anas platyrhynchos domesticus (Itik), the researchers sent samples of the 8-day-old duck eggs to the Institute of Biology, University of the Philippines - Diliman.

Research Locale

The experimental study was conducted in the laboratory of the School of Allied Health, National University- Mall of Asia, using available materials and equipment to complete the whole experiment. The laboratory's location provides convenience for researchers, facilitating easy access to everything required to experiment effectively. The accessibility of the laboratory enhanced the efficiency of the research process and ensured that the experiment proceeded smoothly without unnecessary delays or complications.

Data Gathering Procedure

Preparation of Saccostrea cucullata (Sisi) extract via ethanolic extraction method.

The oysters were washed with water to remove dirt and debris from the shell, and hot water was then poured for open and meat extraction. Oyster meat was shucked using a spoon and boiled in a pot in a 5% salt solution (1:5, w/v) for 1 min. The blanched oysters were dried in a hot air oven at 70 degrees Celsius for 11 hrs. After cooling, the dried oysters were then ground into a fine powder using a blender as well as mortar and pestle (Wang, 2023). After crushing all dried oysters into fine powder, 30g of Saccostrea cucullata powder was weighed and soaked for 48 hrs in 300mL of 95% ethanol placed in a beaker wrapped in aluminum foil and covered with Parafilm at the top of the beaker to avoid contamination. After 48 hrs of soaking, the solution was filtered using Whatman no. 1 filtered paper and concentrated via a rotary evaporator for 1 hour at 60 degrees Celsius and a water bath at 60 degrees Celsius until crude oil emerged (Gamallo, 2016).

Preparation of Duck Anas platyrhynchos domesticus Chorioallantoic Membrane (CAM) Assay.

Regarding the experimental manipulation of Tabamo (2021), researchers used the reference basis for the experiment. The researchers collected three groups of eggs, each consisting of five (5) eggs. Each group included one positive control, one negative control, and three experimental eggs with three different doses (20mg/mL,30mg/mL, and 50mg/mL), a total of 15 eggs. According to Tabamo (2021), dead embryos were discarded to observe embryonic activity. A flashlight was illuminated, marking the air space and chorioallantoic vascula and ture with a pencil. The incubated eggs were then perforated 1x1 cm at the width of the air sac and Chorioallantoic area using a Needle and Sterile Forceps for experimental manipulation. After perforation, the air in

the air sac was drawn using a Rubber Aspirator so that the Chorioallantoic area would have space to administer the extract. The window that was made was covered with a decontamination parafilm (Doctor JPTV, 2020).

Administration of Saccostrea cucullata (Sisi) extract to Duck Chorioallantoic Membrane (CAM) assay

Administration of Saccostrea cucullata (Sisi) extract is then done using a Micropipette directly at the window with varying doses. According to the study by Anglo-Ojeda (2022), ascorbic acid showed promising anti-angiogenesis results at a concentration of 5mg/ml, its peak effect. The 95% ethanol as negative control demonstrated no angiogenic response, confirming that the effect of ethanol in CAM assay is neutral (Bersabal et al., 2022). Therefore, Ascorbic acid was used as the Positive control; the Negative Control had 95% ethanol; 3 eggs in the three experimental groups had corresponding doses of 20mg/mL, 30mg/mL, and 50mg/mL. The three groups of eggs were put in incubation for 2 days at thirty-seven (37) degrees Celsius at sixty-six (66%) percent humidity level (Tabamo, 2021). On the 11th day, the decontamination parafilm was removed, and the window shell was perforated more using sterile forceps to examine the CAM. A trained, licensed professional then observed the CAM. It was observed that pictures were taken using a digital camera on the 11th day to compare controls and treated eggs.

Request for Result Interpretation and Verification of Duck Anas platyrhynchos domesticus Chorioallantoic Membrane (CAM) assay with administered Saccostrea cucullata extract

Researchers requested a trained, licensed professional to interpret and verify whether or not there will be inhibition of Angiogenesis on Anas platyrhynchos domesticus (Itik) Chorioallantoic Membrane (CAM) with administered Saccostrea cucullata (Sisi) extract.

Ethical and Safety Considerations

Safety considerations and ethics were paramount when researching potentially harmful microorganisms and bioactive compounds that researchers may come in contact with within the laboratory. IACUC did not apply to this research study. In the context of investigating the Antiangiogenic Effect of Saccostrea cucullata (Sisi) extract in Varying Doses to Anas platyrhynchos domesticus (Itik) Chorioallantoic Membrane (CAM) Assay, several safety and ethical considerations needed to be followed:

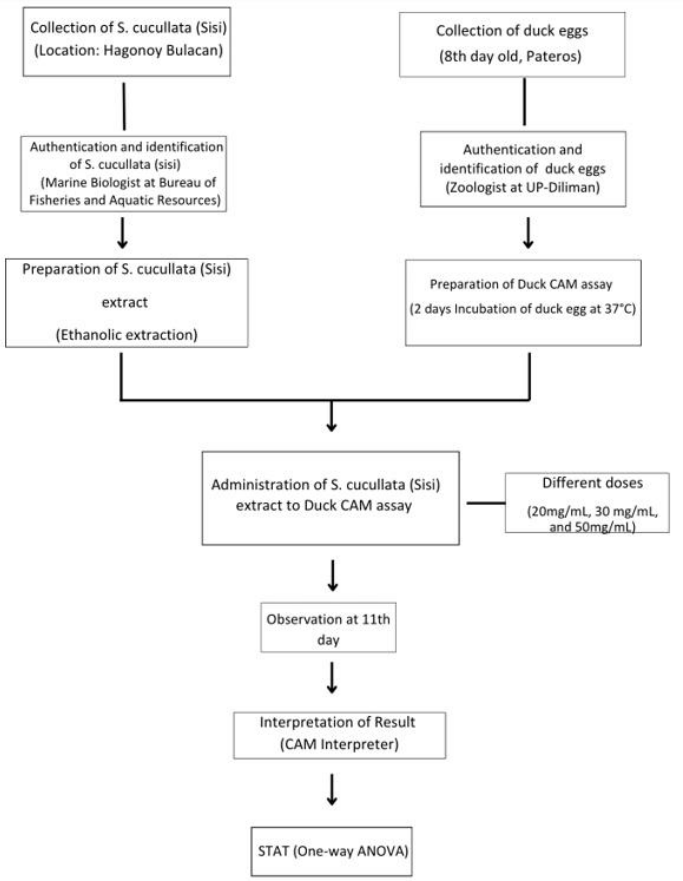
Laboratory Safety Protocols: Researchers adhere to strict laboratory safety protocols to prevent accidental exposure to allergic reactions to Saccostrea cucullata (Sisi). The experiment includes wearing appropriate Personal Protective Equipment (PPE) such as gloves, lab coats, and safety goggles, as well as practicing proper aseptic techniques to minimize the risk of contamination.

Safety Handling: Saccostrea cucullata (Sisi) extract was derived from natural sources but may pose risks if mishandled. Researchers know of potential allergic reactions or adverse effects caused by contact with either extract and take appropriate precautions during handling and storage.

Waste Disposal: Proper disposal procedures were followed to prevent environmental contamination and minimize the risk of spreading infectious agents, including proper disinfection of laboratory equipment and waste materials that come into contact with the specimens used in this study.

Treatment of Data

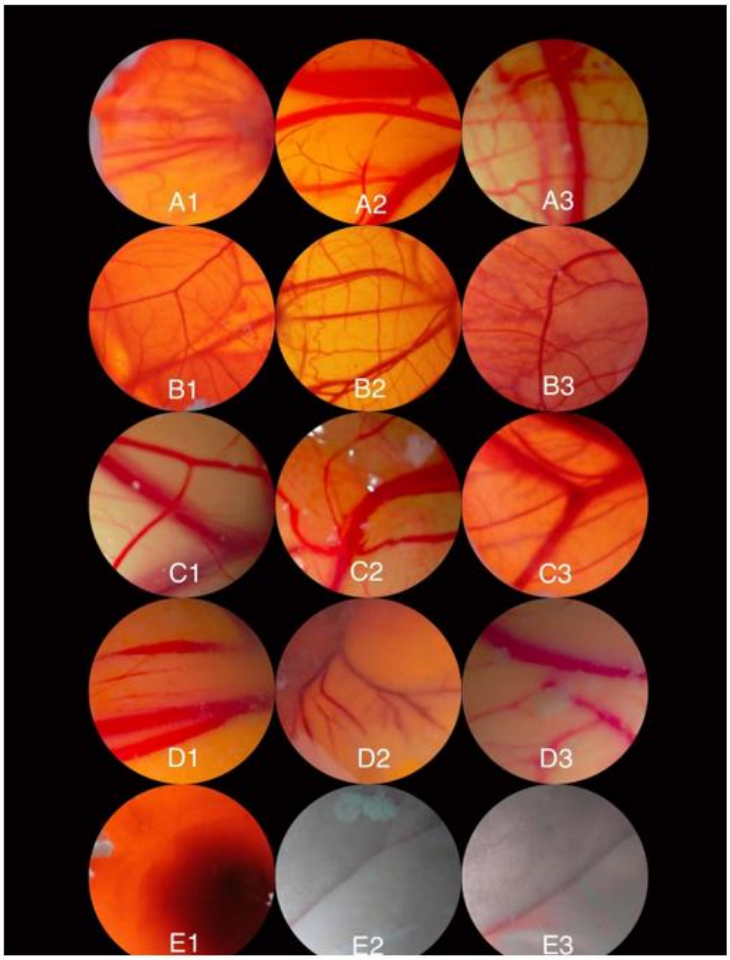
The statistical treatment used in this study was a one-way Analysis of Variance (ANOVA) wherein the researchers had three groups of eggs, each group with five eggs used for Anas platyrhynchos domesticus (Itik) Chorioallantoic Membrane (CAM) Assay. The group of eggs was identified as the negatively controlled eggs, Eggs with positive control, and the eggs administered with different doses of Saccostrea cucullata (Sisi) extract.



144 **Research Flowchart**

145 **Results and Discussion**

Researchers utilized ANOVA to determine the significant effects of Saccostrea cucullata (Sisi) extract on various



dosages (20mg/mL,30mg/mL, and 50mg/mL) on Anas platyrhynchos domesticus (Itik) Chorioallantoic Membrane.

Figure 3. Visual examination

Figure 3. Visual examination of the setups A, B, C, D, and E as the experimental. (A) Positive control, (B) Negative control, (C) 20 mg/ml, (D) 30 mg/ml, and (E) 50 mg/ml.

Table 1. Vascular density of Saccostrea cucullata (Sisi) extract using CAM Assay

Trial	Counted blood vessels trial 1	Counted blood vessels trial 2	Counted blood vessels trial 3
20 mg/ml of *Saccostrea cucullata*	20	22	25
30 mg/ml of *Saccostrea cucullata*	12	15	14
50 mg/ml of *Saccostrea cucullata*	1	2	2
Negative Control (Ethanol)	74	80	78
Positive Control (Ascorbic acid)	45	55	60

Table 1 shows the data from the Anti-Angiogenesis assay conducted with the extract of Saccostrea cucullata on Anas platyrhynchos domesticus and shows the efficacy of various dosages on inhibiting Angiogenesis. This research used 20mg/mL, 30mg/mL, and 50mg/mL. The results in each case were compared against positive and negative controls. These measurements indicate a span of responses;the effect is at best in higher doses; precisely, about the positive control group, these results: Trial 1 obtained 45, Trial 2 obtained 55, and Trial 3 obtained 60, which is far below that in the negative control group which obtained 74, 80, 78 from Trial 1, 2, 3 respectively, thereby affirming that the antiangiogenic activity of the standard treatment used was within expectations. In the case of the extract, it is shown to have increasing effectiveness with higher doses; 50mg/mL was the dosage that exhibited the highest average inhibition. Trial 1 obtained 1, and Trials 2 and 3 obtained 2. Comparing inhibition rates among treatments,raw data across trials gave the lowest for the 50mg/mL treatment compared with the 20mg/mL and 30mg/mL treatments. The inhibition scores with treatments of 20mg/mL and 30mg/mL were lower and more homogeneous in this model;thus,these doses were considered more potent in inducing an antiangiogenic effect than positive control. These data thus indicate that higher doses have a more potent inhibition rate. The minimum dose showing a significant antiangiogenic effect in this experimental setup was required at 50mg/mL.

The highest angiogenesis inhibition was observed in the 50 mg/mL dose of Saccostrea cucullata (Sisi) extract, as presented in Table 1, whereby mean inhibition scores were significantly lower than those of both positive and negative controls. On trial 1 for the 50 mg/mL group, it indicated a mean inhibition score of 1, while on trials 2 and 3,they all recorded 2, indicating vigorous antiangiogenic activity. The results are sharply contrasted by the negative control group, which had mean inhibition scores of 74, 80, and 78 for trials 1, 2, and 3,respectively. According to Tabamo (2012),the CAM treated with the highest extract concentration has the least branching points and faster inhibition. The branching of veins is visible in the negative control, administered with ethanol. Thus, Saccostrea cucullata has a significant effect on reducing vascularization.

Table 2. Significant effect from varying doses of Saccostrea cucullata (Sisi) extract and contact group on Anas platyrhynchos domesticus (Itik) Chorioallantoic Membrane (CAM) Assay

Variables	Source of Variation	Sum of Square	Degree of Freedom	Mean Square	F-statistic	P-value	Interpretation
Effect of *Saccostrea cucullata* (Sisi) extract at varying doses	Between Groups	11538	4	2884.5	188.12	<0.001	Significant
	Within Groups	153.33	10	15.33			

Table 2 shows that the extract has a statistically significant effect on Angiogenesis. The source of variation between groups shows a sum of squares of 11,538 with a degree of freedom of 4, giving a mean square of 2,884.5. This results in an F-statistic for the variation between the two groups of 188.12 with a p-value of less than 0.001. The very low p-value indicates that the obtained differences in variance for the various doses of the extract are significant. Different doses of the extract affect Angiogenesis differently. The results are further supported by a large F-statistic of 188.12, indicating a large between group and within-group variance ratio. In other words,this large ratio could suggest that variation in antiangiogenic effects is due mainly to these different dosages of the extract and less to random fluctuations within the groups. The significant effect of the extract across varying dosages supports the meaning that different levels of the extract significantly impact the inhibition process of Angiogenesis in the CAM assay. From the results of this ANOVA analysis,there is an indication that the extract Saccostrea

cucullata (Sisi) reduced Angiogenesis. Generally, significant differences occurred between the different dosages. s. Its statistical significance at variable doses makes this extract a promising therapeutic agent. Results suggest that the extract of Saccostrea cucullata (Sisi) has a dose-dependent effect on Angiogenesis, indicating good evidence of its efficacy in the experimental model. Therefore,this inference supports further investigation into therapeutic applications of the extract in conditions where Angiogenesis is a key factor. Notably, 50 mg/mL of Saccostrea cucullata (Sisi) extract used in this study is consistent with what was discovered by Tabamo (2021) regarding the Angiogenic Modularity Activity of Sandoricum koetjape (Santol) ethanolic leaf extract. Tabamo (2021) observed that increased concentrations of the S. koetjape extract resulted in a significant reduction of vascular branching points in CAM assay, which shows that these extracts are effective against Angiogenesis. Similarly,the researchers identified that the Saccostrea cucullata (Sisi) extract at 50 mg/mL produced the most pronounced antiangiogenesis activity due to low average inhibition scores and fewer vascular branches present. Therefore, it has been established that extracts from marine sources and plant materials can inhibit Angiogenesis, especially when developing new anticancer drugs since they do not allow blood vessel growth.

Summary of the Findings

The study demonstrated that the Saccostrea cucullata (Sisi) extract showed a significant effect through inhibition of Angiogenesis in the Chorioallantoic Membrane (CAM) of Anas platyrhynchos domesticus (Itik) embryos, with more noticeable effects at higher doses, precisely 50 mg/mL dose exhibited the most significant inhibition than of the other lower doses,thus, indicates a dose dependent response. Using ANOVA as statistical analysis,the findings further confirmed that the differences between various doses were significant, with a p-value of <0.001 and a high F-statistic of 188.12, suggesting that these differences were primarily caused by varied doses rather than random variation. The study results suggest that Saccostrea cucullata extract significantly inhibits Angiogenesis, particularly at higher doses, providing a strong basis for further investigation into its potential therapeutic applications.

Conclusions

This investigation reveals that Saccostrea cucullata (Sisi) extract clearly shows a significant ability to inhibit Angiogenesis and the formation of new blood vessels. The most pronounced antiangiogenic effect was observed at higher doses, particularly at 50mg/mL, showing the most substantial inhibition of blood vessel formation in Anas platyrhynchos domesticus (Itik) Chorioallantoic Membrane (CAM) Assay. The study results indicate that the efficacy of the Saccostrea cucullata (Sisi) extract increases depending on doses. Thus, this demonstrates a dose-dependent relationship. The dose-dependent relationship means that the more extract administered,the greater the angiogenic inhibition effect was observed. The significance of the results was furthermore confirmed with statistical analysis wherein a very low p-value of <0.001 indicates that the differences between doses of Saccostrea cucullata (Sisi) extract were not by chance. Moreover, the F-statistic, which had high results of 188.12,reinforced that the variation of effects in angiogenic inhibition was significantly due to different extract doses, as opposed to random variation within the groupings. These findings suggest that Saccostrea cucullata (Sisi) extract could be a promising candidate for therapeutic uses, particularly in angiogenesis inhibition, which is vital as a treatment for cancer, where the inhibition of blood vessel growth can prevent tumor spread and growth. Furthermore, it will provide a strong basis for further research, specifically on how the extract can be used in medical treatments, emphasizing its potential benefits.

Recommendations

This study recommends additional investigation to understand the mechanisms by which Saccostrea cucullata (Sisi) extract shows angiogenic inhibition, as this could provide insights into its potential supplementary alternative to prevent tumor growth. It suggests optimizing the dosage through further studies to maximize antiangiogenic effects while minimizing side effects. The findings support the need for advanced animal studies and eventual clinical trials to assess the extract's safety and efficacy in humans, particularly in treating conditions like tumor development. The

potential for using the extract in combination with existing antiangiogenic therapies should also be explored to determine if there are synergistic benefits. The study emphasizes the importance of developing effective formulations and delivery methods to ensure the extract reaches target tissues in therapeutic concentrations. It also highlights the significance of developing a before and after effect basis of comparison for better results interpretation. Additionally, it is recommended that future studies set a standard baseline for the usual number of blood vessels observed in a duck egg by conducting a preliminary study or reviewing existing literature. This baseline provides a reference to which comparison can be made on experimental results, helping to determine whether the changes observed in blood vessel formation show positive or negative outcomes. Finally, it calls for comprehensive safety and toxicity assessments to establish safe dosage ranges and evaluate any potential long-term side effects of the extract.

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