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Antiangiogenic Analysis of Selected Medicinal Plants Found in Pampanga, Philippines

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Abstract

Angiogenesis is the process through which blood vessels develop from the pre-existing vasculature. It occurs in both health and sickness throughout life, starting in utero and continuing until old age. Dracontomelon dao, Terminalia catappa, and Ziziphus talanai were examined for their potential to inhibit angiogenesis. Egg candling was used to determine the fertilized eggs. In order to identify the position of the blood vessels, each was marked with a circle using a pencil. Five groups of samples were created and triplicates of the administration were completed. Using the program ImageJ, the suppression of blood vessel creation was assessed. The membranes were captured on camera and examined up close to make comparisons with the initial observation. One-way ANOVA was used to assess the variance between concentrations, and the significant differences were examined using Tukey's post-hoc test ($p < 0.05$). As a confirmation test for the results of the CAM assay quantification, the Drabkin reagent test was conducted. The study found that Dracontomelon dao, Terminalia catappa, and Ziziphus talanai contain phytochemicals like anthraquinones, alkaloids, and flavonoids. However, neither plant extracts showed antiangiogenic properties in the chorioallantoic membrane assay. The D. dao treatment showed significant angiogenesis inhibition at 50µg/mL, while the Z. talanai treatment showed significant differences at 100µg/mL. Based on the analysis of variance, the study found that neither plant extract had the best antiangiogenic potential. The study's results suggest that other factors, such as duck egg days, incubation duration, humidity, and temperature, could contribute to the rotting of eggs.

1. Introduction

Blood vessels form from the existing vasculature through a process known as angiogenesis. Beginning in utero and continuing until old age, it happens in both health and disease throughout life. A blood capillary, which is created by the angiogenesis process, is never more than a few hundred micrometers away from any metabolically active tissue in the body. All tissues require capillaries to facilitate the diffusion exchange of nutrients and metabolites. Angiogenesis and capillarity fluctuate proportionately in response to variations in metabolite activity. In this regulation, oxygen is crucial. For vascular networks to survive and vessel walls to adapt structurally, hemodynamic variables are essential. Over the past 40 years, there has been a lot of interest sparked by the realization that controlling angiogenesis may have therapeutic benefits. In the treatment of peripheral artery disease, ischemic heart disease, and wound healing, angiogenesis can be stimulated. Rheumatoid arthritis, cancer, and other illnesses can all benefit from reducing or inhibiting angiogenesis. In healthy tissues, capillaries develop and contract in response to functional needs. In skeletal muscle and the heart, exercise encourages angiogenesis. Capillary regression is brought on by a lack of exercise. Adipose tissue contains capillaries that expand during weight increase and contract during weight decrease. Without a doubt, angiogenesis continues throughout life [1].

This has prompted the creation of pharmacological anti-angiogenesis drugs, principally by blockage of VEGF/VEGFR signaling, to interrupt the vascular supply and starve the tumor of nutrition and oxygen. 11 anti-VEGF medications have been licensed as a result of a study by Ramjiawan et al., for the treatment of specific advanced malignancies, either alone or in combination with chemotherapy or other targeted therapies. However, this breakthrough rarely had long-lasting effects and had little effect on the overall survival of cancer patients [2]. Combinations of

immune checkpoint blockers and antiangiogenics have gained appeal given the recent success of immunotherapies. However, putting such combinations into practice will call for a deeper mechanical comprehension of how they interact. Because pro-angiogenic factors are overexpressed in tumors, their vasculature is frequently convoluted and a mess, with overly branching leaky arteries.

This increases vascular permeability, which is linked to increased interstitial fluid pressure, decreased blood perfusion, and reduced oxygenation. By reducing vascular permeability and enhancing tumor perfusion and blood flow, anti-angiogenic therapy can temporarily normalize the tumor vasculature and work in conjunction with immunotherapy during this time period. Contrarily, antiangiogenics have the potential to severely prune tumor vasculature in a dose- and time-dependent manner, leading to hypoxia and immunosuppression as well as elevated expression of the immune checkpoint protein PD-L1 [2].

The most fruitful source of leads for the creation of medications has been natural products. More than 100 novel products, primarily as anti-infective and anti-cancer medicines, are currently undergoing clinical trials. Combinatorial chemistry methods are based on natural product scaffolds to create screening libraries that closely resemble drug-like compounds, and the use of molecular biological techniques is expanding the availability of novel compounds that can be conveniently produced in bacteria or yeasts. Data mining and virtual screening methods are also being applied to databases of natural goods in an effort to increase the simplicity with which natural products can be employed in drug development efforts. It is believed that the drug development process will be enhanced by the more effective and efficient application of natural products [3].

Contrary to chemical medications, plants have multiple active chemical components present at once, and because of this, they can influence multiple areas of disease pathology concurrently. In other words, plant extracts rich in biologically active chemicals can inhibit angiogenesis, which prevents metastasis by causing cancer cells to undergo apoptosis, while also slowing the proliferation of cancer cells. The immune system may have the chance to recognize and react to the tumor cell as a result of interactions between the active components in plant extracts and malignancies [4]. Hence, antiangiogenesis is deemed beneficial for humans and plants alike.

Folkman postulated in 1971 that anti-angiogenic therapy might be useful for the treatment of cancer because it could break already-existing blood vessels and prevent the development of new ones, reducing the oxygen and nutrition supply to cancer cells and, as a result, slowing tumor growth. Antibodies like bevacizumab, the first VEGF-targeted drug approved by the Food and Drug Administration (FDA) for cancer treatment, became accessible for cancer therapy decades after Folkman made his statement. Neo-angiogenesis is crucial for the development of tumors and the spread of cancer. Although there are other signaling pathways that are involved in angiogenesis, the VEGF/VEGFRs interaction has been considered to be a key regulator and to be a central target for the development of anti-angiogenic drugs. However, blocking the VEGF signaling pathway with neutralizing antibodies to VEGF or to VEGFRs, soluble VEGFR hybrids, or inhibitors of VEGFRs tyrosine kinase (RTKi) appears to be ineffective Therefore, overcoming resistance brought on by adaptive and compensatory mechanisms is the main difficulty in VEGF-targeted therapy [5].

The findings of a study by Wen et al., show that by controlling the expression of several cytokines and growth factors, such as TNF-, IL-6, IL-1, VEGFA, and TGF-, *D. dao* administration expedites the prompt advancement of infectious wound healing [6]. It helps the development of an infectious wound and lowers angiogenesis and inflammation. The mechanistic analyses show that *D. dao* inhibits the MAPK and PI3K/Akt signaling pathways in addition to NF-B signaling. And that is all, *D. dao* has demonstrated promise in the treatment of cutaneous wounds in infectious wounds, and it has the potential to serve as a novel agent for expediting the healing of infectious wounds in space.

Moreover, a study by Lontoc et al., demonstrates that *Terminalia catappa* leaf extract inhibits angiogenesis on the chorioallantoic membrane of chick embryos at concentrations of 50 ppm, 100 ppm, 200 ppm, and 300 ppm, with a significant difference in angiogenesis between concentrations of 50 ppm, 200 ppm, and 300 ppm. Using a formula derived from the conversion factor (1 ppm = 1mg/L), which converts milligrams of extract to grams and liters to 100 milliliters of distilled water, the concentrations 50 ppm, 100 ppm, 200 ppm, and 300 ppm were prepared from the obtained crude leaf extract of *Terminalia catappa*. As its concentration rises, the tiny blood vessel reduction also rises [7].

Furthermore, a study by Reyes et al., stated that in San Remegio, Antique, Philippines, the Balakat tree (*Ziziphus talanai*) has been used as a traditional remedy for scabies brought on by the parasite mite *Sarcoptes scabiei*, ringworm, and urinary tract infections, or UTI [8,9]. Furthermore, despite the fact that *Ziziphus talanai* bioactive chemicals exhibit activity against bacteria, most investigations on the plant extract were conducted exclusively in vitro. As a result, these effects were not directly observed inside living creatures. Numerous phytochemicals that have the ability to shield the liver and seminiferous tubules against toxicity have been found in the *Ziziphus genus*, according to studies. The phytochemical analysis of *Ziziphus talanai* leaf extract revealed the presence of numerous flavonoids, saponins, and tannins with potential histoprotective properties, as well as trace amounts of alkaloids, triterpenes, and glycosides. Sterol concentrations were also moderate. Therefore, additional research utilizing animal models is required to examine the effects of *Ziziphus talanai* on particular organs.

Using the leaf extracts of Dracontomelon dao, Terminalia catappa, and Ziziphus talanai, this study aims to:

1. Identify the properties present in the selected medicinal plants using phytochemical screening.
2. Evaluate the angiogenic properties of the selected medicinal plants using the chorioallantoic membrane (CAM) assay.

3. Evaluate which selected medicinal plant has the best antiangiogenic potential using analysis of variance (ANOVA).

Future researchers may benefit from this study because it may be utilized to create pharmaceuticals based on the plants' possible antiangiogenic properties. The local government units will also benefit from this research because they will be aware of the medicinal plants' properties, which could lead to the creation of legislation for their preservation as well as more readily available and affordable medical care in the near future.

2. Review of Related Literature

2.1. Plant biodiversity

A major priority for the global human population is the conservation of plant biodiversity. An increase in the number of vulnerable species can be attributed to the significant effects of anthropogenic pressure, foreign species introduction, domesticated species, and chronic weed infestation on plant diversity. The food and medical industries naturally obtain goods from plant biodiversity. Different fundamental raw materials are provided, and it also helps to provide new genetic information that is helpful for breeding programs and the development of crops that are more productive and plants that are more resilient to biological and environmental challenges.

Both in situ and ex situ methods can be used to conserve plant biodiversity. The preservation of plant species in their natural habitats as well as the preservation of domesticated and cultivated species on farms or in their natural environments are examples of in situ techniques. However, due to habitat destruction and the alteration of these natural habitats, there is a significant loss or decline of species, populations, and ecosystem composition, which can result in a loss of biodiversity; thus, in situ approaches alone are insufficient for rescuing endangered species. Plant biodiversity preservation projects are supplemented by additional strategies such as seed bank storage, field gene collections, in vitro collections, and botanical gardens.

They fall within the category of ex situ tactics, which refers to the preservation of biological material away from its native environments. Ex situ conservation is an effective method of preventing the extinction of plants, and in certain situations, it is the only means to protect a particular species. Ex situ and in situ techniques can be used together and are not mutually exclusive. They provide various conservation options, but choosing the best course of action should be dependent on a variety of factors, including the biological makeup of the species and the viability of implementing the selected techniques. In vitro culture and molecular biology-related advancements in plant biotechnology, in particular, have given scientists strong tools to assist and enhance plant diversity management and conservation.

Currently, endangered, rare, crop decorative, medicinal, and forest species are being preserved through biotechnological technologies. This enables the preservation of pathogen-free material, elite plants, and genetic diversity for the short-, medium-, and long-term. For vegetatively propagated and unconventional seed plant species, in vitro preservation is crucial. Additionally, in vitro techniques allow for the safe exchange of plant material across international borders, the construction of large collections in a small amount of space, the provision of essential resources for the recovery of wild populations, and the facilitation of molecular research and ecological studies [10].

2.2. Natural Products

Since ancient times, the use of natural items as a source for treatments has been acknowledged. Despite significant advancements in science and technology, medications generated from natural products continue to play a significant role in drug discovery today. Due to the enormous chemical diversity present in the millions of species of plants, animals, marine organisms, and microbes, nature is a desirable source of new therapeutic candidate chemicals. The impact of evolution on the selection and conservation of self-defense mechanisms, which represent the tactics used to fend off or eliminate predators, is shown in the chemical variety of many living creatures.

There are challenges that must be overcome when creating novel agents from natural sources instead of synthetic ones. For instance, it could be challenging to access the samples' source, to get adequate amounts of the sample, to identify and isolate the sample's active ingredient, and to synthesize the required concentrations of the target chemical. When the tubulin-interacting drug Paclitaxel was first used in clinical settings, these issues were made clear. Initial anticancer activity was seen in ovarian and breast adenocarcinomas among other cancer types. A limited source of the chemical, Paclitaxel was first isolated from the bark of the yew tree *Taxus brevifolia*. Meanwhile, a semi-synthetic counterpart known as docetaxel required some time to create since it was derived from a renewable source, the leaves of *Taxus baccata*.

The current state of both agents' complete synthesis means that drug supply is no longer an issue. Over 60% of medicines that have been licensed for use come from natural sources, according to an analysis of the various chemotherapeutic agents and their origins. As a result of improvements in deep-sea collecting and aquaculture technologies, many chemicals produced from marine species are now being evaluated as potential anticancer drugs in preclinical and early clinical stages. This study briefly discusses a variety of anticancer substances generated from natural products that are under late preclinical development and early clinical trials [11].

All cultures have used plants as a source of medicine and for health benefits since the beginning of time. It is considered that a significant portion of traditional therapy involves the use of plant extracts or their active principles. It has been estimated that between 80 and 85 percent of the world's population relies on traditional medicines for their primary health care needs. Although many recent studies have been conducted to increase the treatment and control of cancer growth, much work and opportunity for improvement still needs to be done. The accompanying adverse effects of synthetic medications are their principal drawbacks.

However, natural treatments including using plants or items made from plants are helpful to fight cancer. In the 1950s, when vinca alkaloids such as vinblastine and vincristine were discovered and developed, as well as when cytotoxic podophyllotoxins were isolated, the hunt for anti-cancer drugs from plant sources began [12].

2.3. Present-Day Cancer Treatment Using Phytochemicals

Humans are given medicinal plants by nature to aid in their quest for improved health. Since ancient times, people have used plants and their bioactive substances as medicines. Numerous medicinal plant species and the phytochemicals they contain prevent the growth and spread of cancer. According to studies, there are over 250 000 plant species in the plant kingdom, but only about 10% of those have been looked at for potential illness treatments. Plant elements such as the flower, flower stigmas, pericarp, sprouts, fruits, seeds, roots, rhizomes, stem, leaf, embryo, and bark all contain phytochemicals and their derived counterparts, which have a variety of therapeutic uses.

Several plant compounds, including alkaloids, flavonoids, lignans, saponins, terpenes, taxanes, vitamins, minerals, glycosides, gums, oils, biomolecules, and other primary and secondary metabolites, play important roles in either inhibiting cancer cell activating proteins, enzymes, and signaling pathways [Cdc2, CDK2 and CDK4 kinases, topoisomerase enzyme, cyclooxygenase and COX-2, Bcl-2, cytokines, PI3K, Akt, MAPK/ERK, MMP, TNK, mechanistic target of rapamycin (mTOR), or by activating DNA repair mechanism (p21, p27, p51, and p53 genes and their protein products), Bax, Bid, and Bak proteins, stimulating the formation of protective enzymes (caspase-3, 7, 8, 9, 10, and 12), and inducing antioxidant action (antioxidant enzymes eg. GSH, GST, and GPxn), thus demonstrating considerable anticancer effects in terms of their effectiveness on the aforementioned proteins, enzymes, and signaling cascades [13].

Vascular endothelial growth factor is a key pro-angiogenic factor for flavonoids. It primarily acts on cells by stimulating two tyrosine kinase receptors, VEGFR1 and VEGFR2, with VEGFR2 being the primary VEGF receptor on the surface of endothelial cells. Numerous studies have shown how important VEGFR2 is for the neovascularization, development, and metastasis of tumors. When VEGFR2 is activated, several downstream signals are phosphorylated, including protein kinase B (AKT), p38 mitogen-activated protein kinases (p38 MAPK), phosphoinositide 3-kinase (PI3K), and extracellular signal-regulated kinases (ERK 1⁄2). These signals are then used to activate endothelial cells, resulting in their proliferation, migration, or tube formation. The VEGF and VEGFR signaling pathways are desirable targets for anticancer therapies, as was previously mentioned. Moreover, a number of studies have shown that chalcones and flavonoids can interfere with VEGF-related cell signaling in cancer or endothelial cells.

Furthermore, fibroblast growth factors (FGFs) in the bFGF signaling pathway can either activate endothelial cell (EC) receptors or trigger the release of proangiogenic molecules from other cells, which in turn stimulates angiogenesis. Furthermore, it appears that resistance to VEGF-inhibitor therapy may be related to dysregulation of FGF signaling. In clinical investigations, a number of compounds have been identified today that disrupt the FGF/FGFR axis. One such instance is the citrus polymethoxy flavonoid nobiletin, which has been shown to block several EC processes, including tube formation, migration, and proliferation. Furthermore, in HUVECs, nobiletin prevented FGF-induced phosphorylation of c-Jun N-terminal kinase (JNK) and extracellular signal-regulated protein kinases 1 and 2 (ERK 1 ⁄ 2). Subsequent research by Liang et al., revealed that kaempferol inhibited the FGF and VEGF signaling pathways, which in turn prevented HUVECs from proliferating and migrating. Furthermore, isoflavones from soy can prevent tumor angiogenesis. Rabiau et al., showed that cancer cells treated with soy isoflavones genistein (40.0 umol/L) have downregulated FGF. As demonstrated by Roy Choudhury and colleagues' study, sorafenib, a multikinase inhibitor with anticancer and antiangiogenic properties, can enhance genistein's capacity to downregulate FGF.

Moreover, hypoxia is a common characteristic of many cancers related to the HIF-1 signaling pathway, and HIF-1 is essential for cells to adjust to decreased oxygen load. Pro-angiogenic factors that it can activate include platelet-derived growth factor (PDGF), VEGF and its receptors, plasminogen activator inhibitor-1 (PAI-1), angiopoietin 1 and 2, the angiopoietin receptor TIE-2 (Tyrosine Kinase with Ig and EGF Homology Domains-2) and MMP-2 and -9. The significance of hypoxia and HIV-1α in tumor angiogenesis and development has made HIF-1α or HIF-1α pathways a promising area of study for anticancer research. *Scutellaria baicalensis's* main ingredient, wogonin, has been shown to suppress angiogenesis by downregulating HIF-1α expression, which in turn reduces VEGF output and suppresses angiogenesis. Numerous mechanisms were proposed, such as enhanced ubiquitination of HIF-1α and its subsequent proteasome destruction, the suppression of HIF-1α's nuclear translocation, or the blockage of Hsp90's binding to HIF-1α. Fu et al. (2016) recently showed that this flavonoid inhibited VEGF, PDGF, and bFGF production and secretion in cancer cells through the c-Myc/HIF-1α signaling axis.

It has been discovered that nobiletin, another flavonoid, inhibits the growth of tumors in vivo as well as the proliferation of cancer cells in vitro. The authors' findings indicate that nobiletin's anticancer properties are linked to its ability to block angiogenesis. A thorough investigation revealed that nobiletin reduced VEGF, AKT, HIF-1α, NF-κB, and other proangiogenic factors. VEGF was suppressed after HIF-1α release was inhibited due to AKT downregulation. As previously indicated, green tea polyphenols may prevent angiogenesis by suppressing VEGF signaling, which is linked to HIF-1α down-regulation. Other authors have also corroborated this suggestion.

Additionally, it was shown that a number of flavonoids inhibited the activity of certain MMPs; it is hypothesized that this effect may have a role in the anticancer and antiangiogenic properties of flavonoids. In our investigation, we discovered that quercetin

reduced HUVECs' release of MMP-2 and MMP-9. The activity of these metalloproteinases was, however, hardly affected by the flavonoids chrysin and 3-hydroxyflavone. Subsequent research by Scoditti et al., revealed quercetin's capacity to lessen angiogenesis induced by phorbol myristate acetate (PMA). The reduction of MMP-9 protein release, gelatinolytic activity, and downregulation of cyclooxygenase 2 (COX-2) expression were linked to the inhibitory impact. Quercetin also inhibits the MAPK and PI3K/AKT signaling pathways, which prevents MMP-2 and MMP-9 from signaling. Finally, thrombospondin-1 (TSP-1) inhibits neovascularization in vivo and in vitro by interacting with endothelial cell surface-expressed CD36 receptors. When this receptor was stimulated, endothelial cell migration and proliferation were inhibited, and apoptosis was induced [14].

Meanwhile, a large range of plants rich in alkaloids have been identified recently, and these substances have a major effect on illnesses that are dependent on angiogenesis. Alkaloids suppress angiogenesis by acting through a variety of ways and possess antiangiogenic properties. When tested on cancer cell lines derived from various histological origins (esophagus, stomach, colon, liver, lung, breast, bone, and brain), almost all alkaloids exhibit antiproliferative and cytotoxic activity. This activity is also dependent on activating the expression of apoptotic genes. However, as was demonstrated for berberine, noscapine, brucine, evodiamine, homoharringtonine, matrine, and tetrandrine, in vitro studies revealed that antiangiogenic effects depend on a shared capacity to downregulate, in the same cancer cells, VEGF, TNF-α, and HIF-1αmessengers and/or protein levels with mechanisms ranging from low expression and higher degradation.

Research has demonstrated, for example, that evodiamine inhibits β-catenin and matrine and tetrandrine block the cascade at the levels of STAT3 signaling. Similar findings were reported for harmine, pterogynidine, sanguinarine, capsaicin, taspine, and taspine's deeper analysis of the molecular pathways showing that Akt phosphorylation, CDK expression, and NF-κB translocation are specifically involved in antiangiogenic activities and that the effect is dose-dependent (10-300 nM). According to additional research, certain alkaloids (such as sinomenine, brucine, and halofuginone) have the ability to directly control the expression of angiogenic factors both in vivo in transplanted mice and in vitro at μM concentrations. Specifically, it should be possible to cause Smad protein depletion with sinomenine and halofuginone.

Very little information is currently available on the role of miRNAs in alkaloid-associated angiogenic processes, despite Ning et al., finding that tetrandrine modulated the expression of miRNAs predicted to be related to wound healing, a process that is closely related to angiogenesis. One exception is berberine, which has been demonstrated to directly stimulate angiogenesis in mice by upregulating miR-29b after ischemia is induced. The remaining data are limited to studies examining alkaloids as mediators of miR induction in various animal or cell models; a recent investigation examined the induction of miRNA by berberine in swine embryos, for instance.

In a similar vein, Huang-Lian-Jiee-Dwu-Tang, a commercially manufactured traditional Chinese medicine, was demonstrated to stimulate the expression of miR-126 and VEGF in mesenchymal stem cell exosomes. This medicine also contained berberine. Interestingly, a recent paper suggests that berberine and evodiamine can influence not only the expression of some miRs (specifically, miR-29a) in colorectal cancer cells, but also the expression of DNA methyltransferases, which in turn control the activities of miRs. This suggests that alkaloids have epigenetic effects during carcinogenesis. In additional investigations, it was demonstrated that the pyrrolizidine alkaloid plant monocrotaline, which causes pulmonary arterial hypertension (PAH), altered the expression of let-7a and miR-21 in the lungs of rats given monocrotaline injections. Human confirmation of miR-21's involvement in PAH was later obtained, although information about miR-21's impact on monocrotaline effects is lacking.

However, the growth-inhibiting alkaloid matrine was also found to downregulate miR-21 on breast cancer cells, which was followed by the dephosphorylation of its target Akt. Alkaloids were found to have a similar effect on human thyroid cancer cells on miR-21. Other miRs, such as miR-19b in melanoma, miR-106b in human acute T-cell lymphoblastic leukemia, and miR-126 in non-small-cell lung cancer, were engaged in different types of cells.

According to Kaymaz et al., in chronic myeloid leukemia cells, capsaicin significantly lowered the expression of miR-520a. The authors postulated that alkaloid's apoptotic capabilities could be directly dependent on the interaction between miR-520a-5p and STAT3, given that the regulation is linked to the reduction of cell proliferation. Similarly, results on rat hepatic stellate T6 cells indicate that miR-15a/16-1 expression may need to be activated for sanguinarine to be able to trigger apoptosis through BCL2 downregulation. Conversely, ectopic expression of homoharringtonine was found to be causally linked to the activation of miR-370 and its target FoxM1, a key regulator of both apoptosis and cell proliferation. Additional transfection experiments using miR mimics indisputably showed that miR-324-5p is necessary for the inhibitory effects of sinomenine on invasion and metastasis of breast cancer cells, which also include downstream NF-κB [15].

Last but not least, emodin is an anthraquinone derived from *Rheum palmatum* L.'s root and rhizome. and discovered in additional plants. There have been reports of antibacterial, anticancer, diuretic, and vasorelaxant properties for this active ingredient. According to reports, EMD prevents MMP production in human neuroblastoma cells and blocks VEGFR signaling in human colon cancer cells, hence preventing tumor-induced angiogenesis and metastasis. On the other hand, it's unclear how EMD influences angiogenesis and metastasis in cases of human breast cancer. As a result, we assessed how EMD inhibits breast cancer metastasis and angiogenesis. It was discovered that this derivative of anthraquinone exhibited noteworthy anticancer activities and enhanced overall survival in cases of human breast cancer. As a result, we assessed how EMD inhibits breast cancer metastasis and angiogenesis. It was discovered that this derivative

of anthraquinone exhibited noteworthy anticancer activities and enhanced overall survival in cases of human breast cancer. Both in vitro and in vivo, it was discovered that EMD reduced the angiogenesis and metastasis caused by tumor cells. Moreover, MMPs and VEGFR-2 inhibition, which may be connected to the downregulation of Runx2 transcriptional activity in breast cancer, were the sources of these EMD inhibitory actions.

Tumor cell migration and inversion are essential components of the metastatic processes. Furthermore, a critical stage in the invasion and migration of tumor cells during metastasis is the extracellular matrix's disintegration by MMPs in the surrounding tissues. It was shown that EMD prevented the invasion and migration of tumor cells in vitro and reduced the establishment of tumor foci in experimental metastasis in vivo. EMD reduced MMP activity and the expression of MMP-9 and MMP-13, according to a western blot assay and FRET-based MMPs study. MMPs are a class of zinc-dependent endopeptidases that are linked both structurally and functionally. There are currently 23 knowns human MMPs, comprising 6 membrane-associated enzymes and 17 soluble, secreted enzymes.

These enzymes play a role in the proteolysis of the extracellular matrix (ECM), the regulation of cell adhesion, migration, and EMT, the processing of growth factors, and tumor-induced angiogenesis, among many other physiological and pathological processes, including embryonic development, wound healing, tumor growth, invasion, and metastasis. According to published evidence, MMPs play important roles in the development and spread of breast cancer. These findings suggested that MMP inhibition may be linked to EMD attenuation of breast cancer spreading characteristics [16].

2.4. Angiogenesis

More than 200 years ago, the Scottish surgeon Dr. John Hunter found that the development of new blood vessels was an essential phase in tissue proliferation. He coined the term "angiogenesis" to describe this process. Prior to Professor E. Goldmann's broad visualization of the creation of blood vessels during tumor progression in 1907, there were, however, few reports of angiogenesis in tumors in the subsequent 100 years. In the late 1930s and early 1940s, experimental studies of angiogenesis were initiated, as opposed to only viewing anatomical specimens. Before the 1970s, it was generally believed that tumor angiogenesis was a byproduct of tumor cells that were perishing.

No tumors can grow larger than 2 mm unless they are vascularized, and tumors can be kept small and put into a latent state by preventing new vessel creation, according to the 1971 theory put forth by Folkman et al. Since then, work on creating medicines for solid tumors have been mostly driven by this "anti-angiogenesis" notion, which advanced after basic fibroblast growth factor (bFGF) and VEGF were discovered by several research groups in the 1980s. Since VEGF demonstrated a critical role in angiogenesis in loss-of-function studies, it was quickly decided that VEGF would be the primary prospective target for the development of anti-angiogenesis agents. The VEGF antibody, which Ferrara's team originally demonstrated in 1993, has served as the cornerstone for the advancement of specific anti-angiogenesis treatments ever since [17].

2.5. *Dracontomelon dao*

Dracontomelon dao is a native tree to the Philippines that belongs to the Anacardiaceae family and is found all throughout South and Southeast Asia. The tree is utilized as firewood and planted as a decoration in plantings along roadways. Cooked and consumed as a vegetable and used to flavor meals, the blossoms and leaves. The tree's bark is used in conventional medicine as a depurative, treatment for dysentery, skin infections, dermatitis, sore throats, and as a general medication for expectant mothers. The wood of the trunk is made into a decoction that locals use as an anti-inflammatory and anticancer agent. Several pharmacological investigations on *D. dao* shown antibacterial, antioxidant, anti-inflammatory, anti-diabetic, and anti-trypanosomal actions, and the isolation of the chemical components of its various portions suggests the presence of medicinal and therapeutic characteristics.

Over the course of 1000 years, it has been utilized to treat a variety of infectious skin problems. There is evidence from earlier accounts that *D. dao* exhibits favorable antibacterial action against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli*. *Dracontomelon dao* (Blanco) Merr has alkaloid, sterol, tannin, terpenoids, and flavonoid components according to chemical analysis. The plant has an antioxidant effect, as demonstrated by the flavonoid's existence and the corresponding qualities in empirical application. In fruits, vegetables, and nuts, flavonoids are abundant. They represent one of the most significant chemical classes of natural chemicals, with pharmacological properties ranging from anti-inflammatory to anti-cancer to cardioprotective to neuroprotective. The most potent types of flavonoids, whose antiangiogenic effect was dose-dependent, were discovered to be isoflavones, flavonols, and flavones [18].

Over the past 20 years, a lot of research has been done on the antiangiogenic properties of flavonoids. By interfering with important angiogenesis signaling cascades like the mitogen activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) pathways, flavonoids have been shown in numerous studies to be able to limit the proliferation and migration of endothelial cells. They can, however, prevent the expression of important proangiogenic molecules like VEGF and matrix metalloproteinases (MMPs). It was also discovered that *D. dao* has an impact on reducing skin tissue histological damage and inflammatory cytokine levels. Additionally, *D. dao* can control the relative protein levels of the MAPK/NF-B and PI3K/AKT signaling pathways, control angiogenesis and inflammation, and alleviate skin tissue injury in *E. coli*-induced wound infection by controlling both the PI3K/AKT and MAPK/NF-B signaling pathways.

Figure 1: *Dracontomelon dao*

2.6. *Terminalia catappa*

Terminalia catappa Linn. (Combretaceae) is a family of trees that grows up to 35 meters tall with horizontal branches and an upright, symmetrical crown. It typically has tier-based branch arrangements. The leaves are large, oval, leathery, glossy, and dark green. They are 10 to 14 cm wide and 15 to 25 cm long. Because the trees are monoecious, they produce separate male and female blooms on different branches. Both flowers are small, 1 cm in diameter, without petals, and white to greenish in color. The fruit is a drupe that measures 5-7 cm in length, 3-5.5 cm in width, and has one seed inside. When ripe, it starts out green, then goes yellow, and finally turns red. The fruit's seed is edible when it is fully ripe. The aforementioned species is native and widely distributed in Southeast Asia [19]. The initial screening of 27 species of Philippine medicinal plants by Taganna et al., gave the direction for further investigation of *Terminalia catappa's* anti-quorum sensing capability. The presence of QS inhibitors in *Terminalia catappa* methanolic extracts was discovered by the inhibition of violacein pigmentation in *Chromobacterium violaceum* growing on agar plates [20].

The word "terminalis," which refers to the leaves clustered at the terminals of the shoots, is where the generic name derives from. It is a sizable tree that thrives in tropical and subtropical environments. It is extensively cultivated all over the tropics, specifically in the tropical and subtropical zones of the Indian and Pacific Oceans. This tree is raised for its delicious nuts which could be consumed raw, as well as for decorative purposes. The plant *T. catappa* is well-known in Ayurveda. Its fresh leaf juice is used to make a leprosy and scabies lotion and is also eaten internally to treat headaches and stomachaches [21]. Different portions of *T. catappa* include a variety of phytoconstituents, according to phytochemical studies. These include para-hydroxybenzoic acid, alkaloids, flavonoids, polyphenols, saponosides, sterols, carbohydrates, proteins, carotenoids (β-carotene), lipids, and vitamins. Among these, phenolic chemicals, tannins, flavonoids, carotenoids, and triterpenes/triterpenoids are widely distributed and are known to cause a variety of useful reactions, including anti-inflammatory, antiangiogenic, antioxidant, hypocholesterolemic, and hypoglycemic ones. Additionally, several researches have suggested that sterols and alkaloids produced from *T. catappa* exhibit comparable characteristics [22].

Figure 2: *Terminalia catappa*

2.7. Ziziphus talanai

In the buckthorn family Rhamnaceae, the genus *Ziziphus* contains roughly 40 species of prickly shrubs and small trees that are found worldwide in warm-temperate and subtropical climates. It only exists on the Philippine islands of Luzon and Visayas, notably in the Philippine Limestone Forest ecoregion. Additionally, it is dispersed in lowland primary or secondary woods up to an altitude of 1000 m. [23]. Some species are deciduous, while others are evergreen. The leaves are alternating, whole, and 2–7 cm long with three distinct basal veins. The flowers are tiny, unassuming yellow-green blossoms. The fruit is an edible drupe that is globose, oblong, or reddish-brown in color, 1 to 5 cm long, and frequently highly delicious and sugary. The *Zizyphus jujuba* Mill species is the most well-known. Other species are *Z. spina-christi* from Southwestern Asia, *Z. lotus* from the Mediterranean, *Zizyphus mauritiana* Lamk. which is distributed from western Africa to India, and *Zizyphus joazeiro* which flourishes in Brazil's Caatinga region [24].

Meanwhile, people have long utilized *Ziziphus talanai* (Blanco) Merr., an endemic species in the Philippines known locally as the Balakat tree, as an herbal remedy to treat parasite infections brought on by ringworm and mites as well as bacterial disorders including urinary tract infections. A methanolic bark extract from the Balakat tree was shown to be efficient against Gram-positive bacteria but ineffective against Gram-negative bacteria and several fungal species, according to groundbreaking research. The crude ethanolic extract of the Balakat tree was shown to have hepatoprotective and reproprotective actions against tetracycline-induced liver damage and reproductive failure in mouse models, in addition to its potential antibacterial properties. Crude extracts of plants belonging to the genus *Ziziphus* have demonstrated successful antibacterial effects against test microorganisms, clinical harmful strains as well as isolates. An earlier phytochemical analysis of the ethanol leaf extract of the Balakat tree found high amounts of flavonoids, saponins, and tannins along with small amounts of triterpenes, alkaloids, and glycosides [25]. *Ziziphus* leaf extracts dramatically reduced VEGF and TGF-1 expression compared to infected untreated and PZQ-treated groups, demonstrating its antiangiogenic and anti-fibrotic actions on granulomas. When compared to the infected untreated group, it significantly lowers the percentage of positive Ki-67 hepatocytes, demonstrating antiproliferative action. Furthermore, when compared to infected untreated and PZQ-treated groups, it demonstrates powerful antioxidant activities, as seen by a significantly lower NO and conservation of hepatic GSH, GST, and SOD in hepatic homogenates [26].

Figure 3: *Ziziphus talanai*

2.8. Chorioallantoic Membrane Assay

The iris and avascular cornea of rodent eyes, the rabbit ear chamber, the mouse dorsal skin and air sac, the chick embryo chorioallantoic membrane (CAM), and the zebrafish are among the traditional assays for examining angiogenesis in vivo. Significant progress has been made in understanding the mechanism of action of many angiogenic factors and inhibitors thanks to in vivo angiogenesis experiments. The cost, usability, reproducibility, and dependability of a procedure are the primary factors that influence the decision. However, in vivo angiogenesis experiments could be extremely susceptible to outside influences and difficult to access for biochemical analysis. Additionally, their interpretation is frequently made more difficult by the possibility that the experimental protocol used favors inflammation.

In this situation, the stimulation of inflammatory or other non-endothelial cell types indirectly induces the angiogenic response, at least in part. The chorion and allantois fused to form the CAM, an extra-embryonic membrane, on day 4 of incubation. Undeveloped blood vessels that are dispersed throughout the mesoderm and lack a complete basal lamina and smooth muscle cells grow quickly until day 8 and give rise to a capillary plexus, which develops

an intimate relationship with the chorionic epithelial cells above it and mediates gas exchange with the environment. At day 14, the capillary plexus is situated next to the shell membrane at the ectoderm's surface.

Up until day 11, there is rapid capillary growth; after that, the endothelial cell mitotic index rapidly decreases, and on day 18, just before hatching, the vascular system is at its final configuration. CAMs are cultivated in Petri dishes and plastic wrap/cup apparatus either in ovo or ex-ovo as a shell-less culture. There is no conclusive proof that the data obtained utilizing the in ovo or shell-less culturing methods differ significantly from one another. The key success limiting factor in this culture approach has been shown to be the survival rate of eggs cultured ex ovo. The most common approach is still to apply test and control compounds locally. It is rapid, semi-quantifiable, affordable, and suitable for screening a variety of novel compounds.

The only drawback to this strategy is the measurement of antiangiogenic medication interactions with CAM vasculature as opposed to pro-angiogenic compounds. In the literature, numerous application techniques or carriers are discussed for testing angiogenic or antiangiogenic action. Small filter disks or fragments of polymerized materials, such as gelatin sponges or synthetic polymers that are physiologically inert, are frequently used to deliver the test sample. The number, diameter, density, permeability, branch point count, and blood flow of blood arteries can all be measured. In order to quantify angiogenesis and antiangiogenesis in the CAM, we have created a novel technique. On day 8, developing CAM are implanted with gelatin sponges either with a stimulator or an inhibitor of blood vessel creation.

On day 12, morphometric counting of blood vessels that are extending vertically into the sponge and at the juncture where the sponge meets the surrounding mesenchyme is done. The newly created blood vessels are measured by morphometric analysis of histologic CAM sections and grow perpendicular to the plane of the CAM within the sponge, which is devoid of preexisting vessels. More advanced methods, such as in OVO cell proliferation, layered expression scanning to see the protein of interest, and fluorescent confocal microscopy of new blood vessel formation at the application site, have been developed recently to perform a reliable quantitative evaluation of vascular density. Antiangiogenesis is thought to be indicated by the emergence of an avascular zone or a zone of inhibition at the application site.

When protamine was administered to the leading edge of the CAM, Taylor and Folkman demonstrated how it created an avascular zone, which is how it was first defined. A well-known angiogenic cytokine, typically fibroblast growth factor-2 (FGF-2) or VEGF, is used to evaluate the inhibition of angiogenic response in studies of angiogenesis. These two approaches differ in the target vessels and are used to examine the response in the rapidly growing CAM blood vessels (Ribatti, 2010).

3. Materials and Methods

3.1. Plant Identification and Collection

A. Plant Identification

The three medicinal plants were identified by the researcher according to their morphological characteristics, such as their height, bark, pollen, leaves, fruit, and flowers.

Dracontomelon dao. Wood weighs about average. Absent ripple marks. Lack of growth rings. diffusely permeable wood. Tangential vessel diameters varied from 260 to 330 um, and there were 5 to 7 vessels per millimeter. The vessels were solitary and in radial multiples of 2 to 3. Simple perforation plates. Pits in the intervessel space are big, polygonal, and alternating, measuring 11 to 13 um. Large, circular, gash-like vessel-ray pits. Present tyloses but no deposits. The septate, thin to thick-walled fibers have a length range of 1400 to 1900 um and are primarily found on the radial walls. The axial parenchyma was aliform and vasicentric. Body ray cells are procumbent and have one row of upright marginal cells. Rays are 2 to 4 cells wide and range in height from 850 to 1100 um. Absence of radial canals. Procumbent, upright ray cells, non-chambered axial parenchyma, and fiber all had mineral inclusions with prismatic crystals. Silica isn't present [27].

Terminalia catappa. It can reach a height of 25 meters and has broad, glossy leaves that change to crimson or yellow before dropping. A little peach-sized edible fruit is produced by the tree. Typically blooming from February to May, its flowers are white or light green. Although it can grow to heights of 30 to 40 feet, cultivating it is thought to be moderately difficult [28].

Ziziphus talanai. The balacat tree may grow to a height of 30 meters and a diameter of 100 cm, and it is distinguished by its tall, straight, cylindrical, and thorny trunk. *Ziziphus* species traits include alternate, whole leaves with three noticeable basal veins, tiny, inconspicuous yellow-green blooms, and drupes that are 1 to 5 cm long.

B. Plant Collection

A letter of permission was given to the corresponding offices in Angeles University Foundation, Mansfield Residences, and Our Lady of Grace Parish prior to beginning the fieldwork in Pampanga, Philippines for the collection of 2 kilograms each of *Dracontomelon dao*, *Terminalia catappa*, and *Ziziphus talanai*.

The plant collection was under the direction of Ms. Sheila S. Cabral and Engr. Carolyn A. Arbotante. The Co's Digital Flora and Stuart X Change online resources were used for preliminary identification to guarantee the accuracy of the chosen indigenous flora that were collected.

3.2. Preparation of Plant Samples

A. Herbarium

The gathered leaf samples were thoroughly washed with tap water. For the sake of future plant identification, an herbarium was made by placing the collected leaf samples on a newspaper and flattening them in order to release excess water and moisture. Afterwards,

the newspapers with the collected plant samples were soaked with 95% ethanol inside a garbage bag for 3 days in order to dry the leaves.

After 3 days, the dried plants were brushed with glue in order for it to stick in the vellum board. Brown tape was used to fasten the thick stems into the vellum board. Afterwards, labels were placed in the bottom right corner of the vellum board, which contains important information such as the botanical name, family, genus, species, local name, place collected, date of collection, and name of collector. Once the labels were placed, the vellum board was placed inside a polyethylene bag and kept for future identification.

B. Crude Extraction

The protocol from Guevara (2005) was used for crude extraction. First, the samples were air-dried to make them brittle before being ground. Then, the materials that had been ground up were weighed and put in a beaker. Afterwards, 85% ethanol was added as an extraction solvent which required the samples to be soaked in it for 24 to 48 hours.

Ethanol was used to extract the biological components, while the Whatman filter paper and glass funnel were used in the subsequent filtering procedure. First, the filtrate was poured in a beaker with sterile gauze on top, which was used to cover the beakers before crude extraction in order to prevent pressure build-up inside. Afterwards, the ethanol in the extract was removed using rotary evaporation in the AUF Center for Advanced Research and Innovation (CARI). The species and date of extraction of the gathered crude extracts was noted on the labels.

3.3. Phytochemical Screening

The plant extracts underwent phytochemical screening to identify the secondary metabolites present before the chorioallantoic membrane (CAM) experiment. The common laboratory practices for phytochemical screening were carried out using the book "A Guidebook to Plant Screening: Phytochemical and Biological" by Guevara (2005) as a reference. Some alterations were done by Tongol [29].

Solvent system preparation. To find the solvent with the best separation, many solvent combinations were prepared for the solvent system. As a result, several solvent system ratios—3:7, 7:3, 6:4, 1:9, and 9:1—were likewise employed and were put into five distinct beakers.

TLC plate preparation. TLC plates of two centimeters by seven centimeters were made, and markings at 0.5 centimeters from the top and 1 centimeter from the bottom were measured and marked. Using a tapered capillary tube, plant extracts were blotted onto the plate. Development chamber preparation. The TLC chamber was lined with filter sheets, and 10 mL of the prepared solvent was added. Chromatogram development. The blotted TLC plates were placed in separate beakers holding the various ratios to be tested in order to determine the best solvent. The blotted region should not be in the solvents. The chambers were kept covered throughout the elution process. The extract with the best spot separation and yielded the best Rf values was chosen to be used after the five TLC plates were observed.

Screening for phytochemicals with spray reagents. The spray method of phytochemical screening was used in the chromatogram and involved three trials. The following spraying reagents were used to examine the secondary metabolites on the blotted TLC plates:

Antimony (III) chloride. On the chromatogram, Antimony (III) chloride was sprayed. If the hue changed from yellow to orange after air drying, flavonoids were present.

Potassium ferricyanide-ferric chloride. On the chromatogram, Potassium ferricyanide-ferric chloride was sprayed. If there is a presence of blue spots on the TLC plate after being treated with hydrochloric acid (HCl), flavonoids are present.

Dragendorff's reagent: On the chromatogram, Dragendorff's reagent was sprayed. If there were brown to orange color changes following spraying, it was determined that alkaloids were present.

Methanolic KOH: If an orange hue appears on the chromatogram, it can be inferred that anthraquinones are present.

Acetic-anhydride sulfuric acid: After spraying Acetic-anhydride sulfuric acid on the chromatogram, it will be examined under visible and UV light (365 nm). If fluorescence or black patches appear on the chromatogram, it can be inferred that steroids and saponins are present.

3.4. Chorioallantoic Membrane Assay

Before the collection of 2-3 trays of fertilized duck eggs from Pikes Gomez Farm in Barangay San Rafael, Tarlac City, an Institutional Animal Care and Use Committee (IACUC) permit from Pampanga State Agricultural University was secured. The methodology by Mousa and Davis then served as the source for the chorioallantoic membrane (CAM) assay with slight modifications. Starting from day 1 to day 8, the eggs were incubated at 37°C with no rotation done. On day 9, egg candling was used to separate and collect the fertilized from the unfertilized eggs. On day 13, the gathered eggs were cleaned with a sterile gauze and 70% ethyl alcohol, and marked with a circle using a pencil in order to identify the position of the blood vessels. On day 14, a 1x1 cm opening was made in the eggs using a sterilized push pin. Afterwards, the treatments were administered and the opening was sealed with scotch tape. Finally, the eggs were incubated again for 24 hours at 37°C before being observed for the purpose of quantifying the blood vessels.

3.5. Administering Treatment

Five group samples were created: positive control group (quercetin), negative control group (distilled water), and three treatment groups which received plant crude extracts at concentrations of 10, 30, 50, and 100 µg/ml. Three trials were made for each group.

The antiangiogenic property was calculated using the following equation:

$$\% AIA = [(X - Y)/ X] 100$$

Where X would represent the number of blood vessels from the untreated CAM, and Y would represent the number of blood vessels from the treated CAM. To provide comparable results, each treatment will be carried out in triplicates.

A flashlight and iPhone 11 smartphone were used to capture images for the quantification of blood vessels present after the treatments. To locate the areas where blood vessels branch out, the images were run via ImageJ, specifically the 'Analyze HUVEC Phase Contrast' function.

3.6. Drabkin Reagent Test

The Drabkin reagent test determines hemoglobin levels in blood and is used to confirm the results of the CAM assay. First, the CAM was crushed, filtered, and added to a 5mL chilled normal saline solution. The solution was then processed for 30 minutes at 2500 rpm. Afterwards, 3mL of Drabkin reagent and 1mL of supernatant were combined, incubated at 27 ± 3 °C for 20 minutes, and measured using a UV spectrophotometer (580 nm). For eight samples, 7 from *D. dao* and 1 from distilled water, further filtration was performed to ensure no particles were present.

3.7. Proper Disposal

The chilled normal saline solution was used to sedate the eggs before the Drabkin reagent test. Then, the embryos were put in color-coded bags for disposal when the aforementioned operation was concluded.

3.8. Data Analysis

Descriptive analysis was used to clarify macroscopic observations. One-way ANOVA was used to assess the variance between concentrations, and the significant differences were examined using Tukey's post-hoc test ($p < 0.05$).

4. Results and Discussions

4.1. Phytochemical Screening

Several phytochemicals were screened for using a qualitative analysis (Table 1; Appendix B). It was found that *D. dao*, *T. catappa*, and *Z. talanai* contains anthraquinones, alkaloids, and flavonoids, according to the spray reagents.

Phytochemicals	*Dracontomelon dao*	*Terminalia catappa*	*Ziziphus talanai*
Flavonoids	+	+	+
Alkaloids	+	+	+
Anthraquinones	+	+	+
Steroids	-	-	-
Saponins	-	-	-

Table 1: Phytochemical Constituents in the Selected Medicinal Plants

These results coincide with the study of Zhumashova et al., who studied the scientific literature, and compared the previously identified compounds in other rhubarbs [30]. The authors noticed that *Rheum cordatum* belongs to the plants with a higher content of phenolic metabolites from anthraquinones. In all analyzed extracts, the authors distinguished these two groups of components as the major ones.

Moreover, the results conform to the study of Agidew who extracted alkaloids from various plant parts using a variety of solvents, including ethanol, methanol, chloroform, acetone, hexane, petroleum ether, ethyl acetate, and aqueous (water). Phytochemical components from medicinal plants, such as leaves, roots, stem bark, and fruits, are extracted using these kinds of solvents [31].

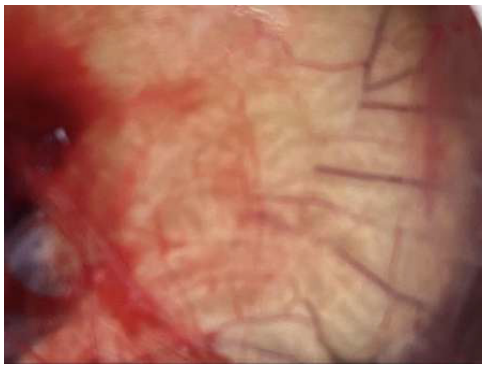
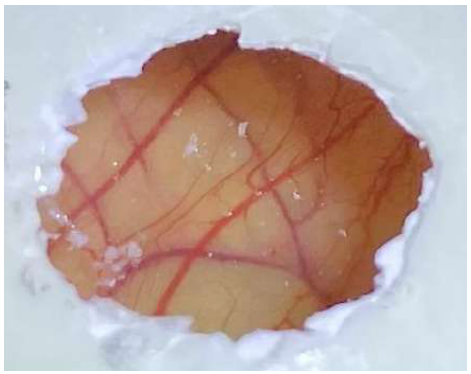
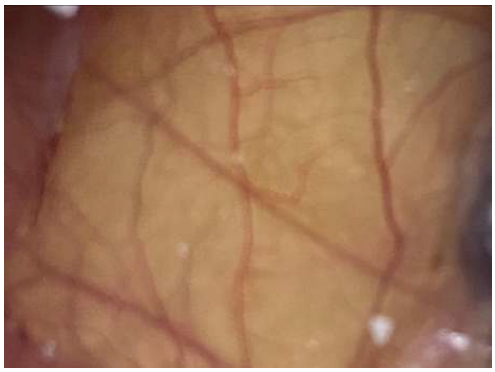
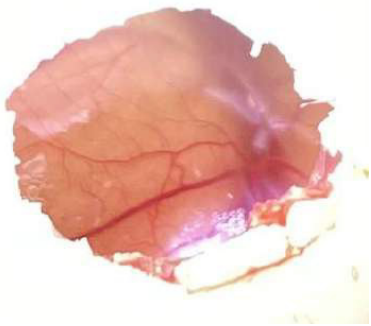
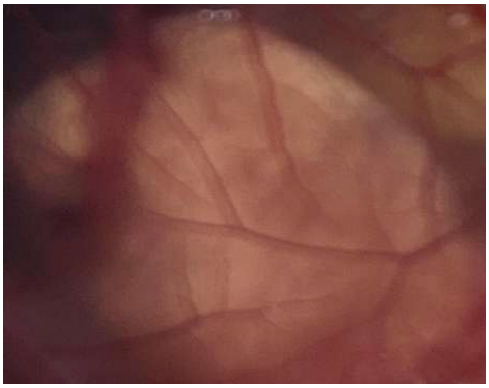
Furthermore, Agidew also found that plants are omnipresent sources of flavonoids, a broad class of polyphenolic chemicals with a benzoyl-γ-pyrone structure. The phenylpropanoid pathway is responsible for their synthesis. Reports that are now available generally indicate that the range of pharmacological activities are caused by secondary metabolites of a phenolic character, including flavonoids. Plants are known to produce flavonoids, which are hydroxylated phenolic compounds, in reaction to microbial infection.

4.2. Chorioallantoic Membrane Assay

After 24 hours, only 16 eggs survived out of 59—8 eggs from Dao, 3 eggs from Balakat, 1 egg from Quercetin, and 4 eggs from distilled water. Using the program ImageJ, the suppression of blood vessel creation was assessed. The membranes were captured on camera and examined up close to make comparisons with the initial observation. Listed below are the summaries of percent angiogenesis inhibition at different concentrations.

Treatments	Trial 1	Trial 2	Trial 3	Mean	S.E.M.
D. dao	-140.28%	-166.67%	-188.30%	-165.08%	±4.73%
Z. talanai	0.40%	-130.11%	0%	-43.24%	±4.73%

Table 2: Summary of Percent Angiogenesis Inhibition At 10µg/ml with The Computed Mean and Standard Error of Mean

Day 14 (Untreated)	Day 15 (Treated)
A	
	
B	
	
C	
	

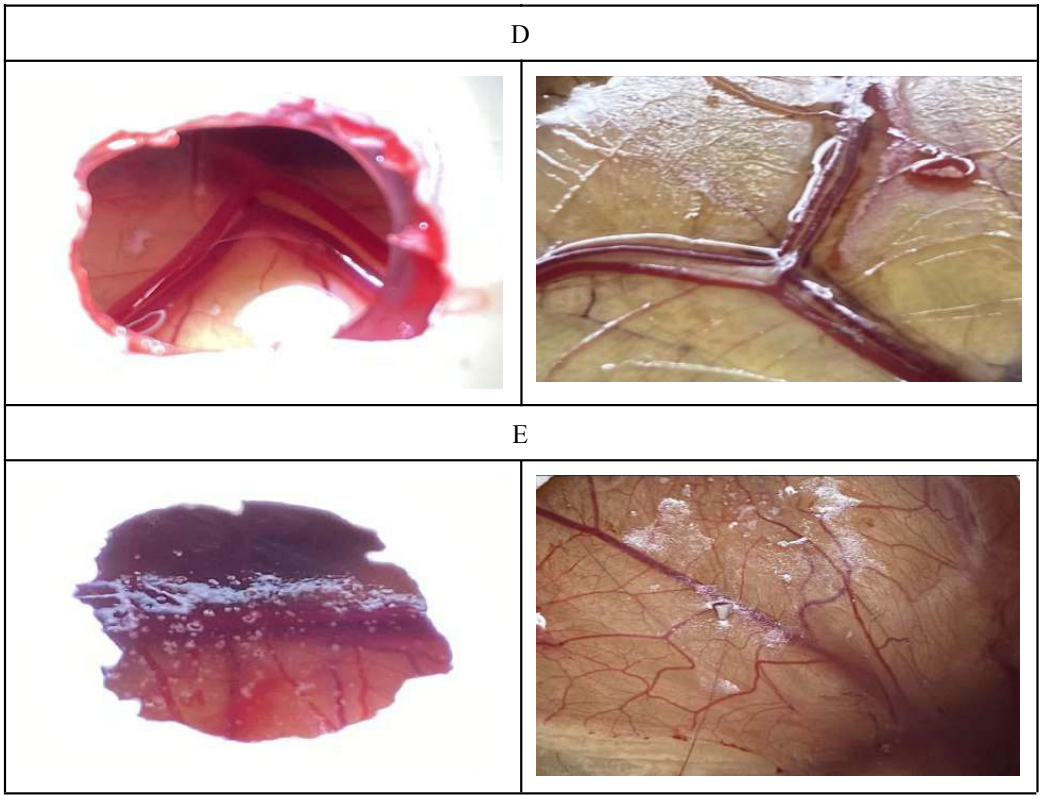


Figure 1: CAM assay treatments at 10µg/mL (A) CAM treated with *D. dao* (-165.08 ± 4.73% of inhibition); (B) CAM treated with *D. dao* (-165.08 ± 4.73% of inhibition); (C) CAM treated with *D. dao* (-165.08 ± 4.73% of inhibition); (D) CAM treated with *Z. talanai* (-43.24 ± 4.73% of inhibition); (E) CAM treated with *Z. talanai* (-43.24 ± 4.73% of inhibition)

At a concentration of 10µg/mL, -165.08 ± 4.73% ($p<0.05$) and -43.24 ± 4.73% ($p<0.05$) were the respective percent angiogenesis inhibition of the *D. dao* and *Z. talanai* plant extracts. We are unable to draw a precise conclusion for the 10µg/mL concentration because no eggs in the control group survived.

Treatments	Trial 1	Trial 2	Trial 3	Mean	S.E.M.
Distilled water	-130.35%	0%	0%	-43.45%	±3.08%
D. dao	-929.17%	90.48%	0%	-279.56%	±3.08%
Z. talanai	-409.65%	0%	0%	-136.55%	±3.08%

Table 3: Summary of Percent Angiogenesis Inhibition At 30µg/ml with The Computed Mean and Standard Error of Mean

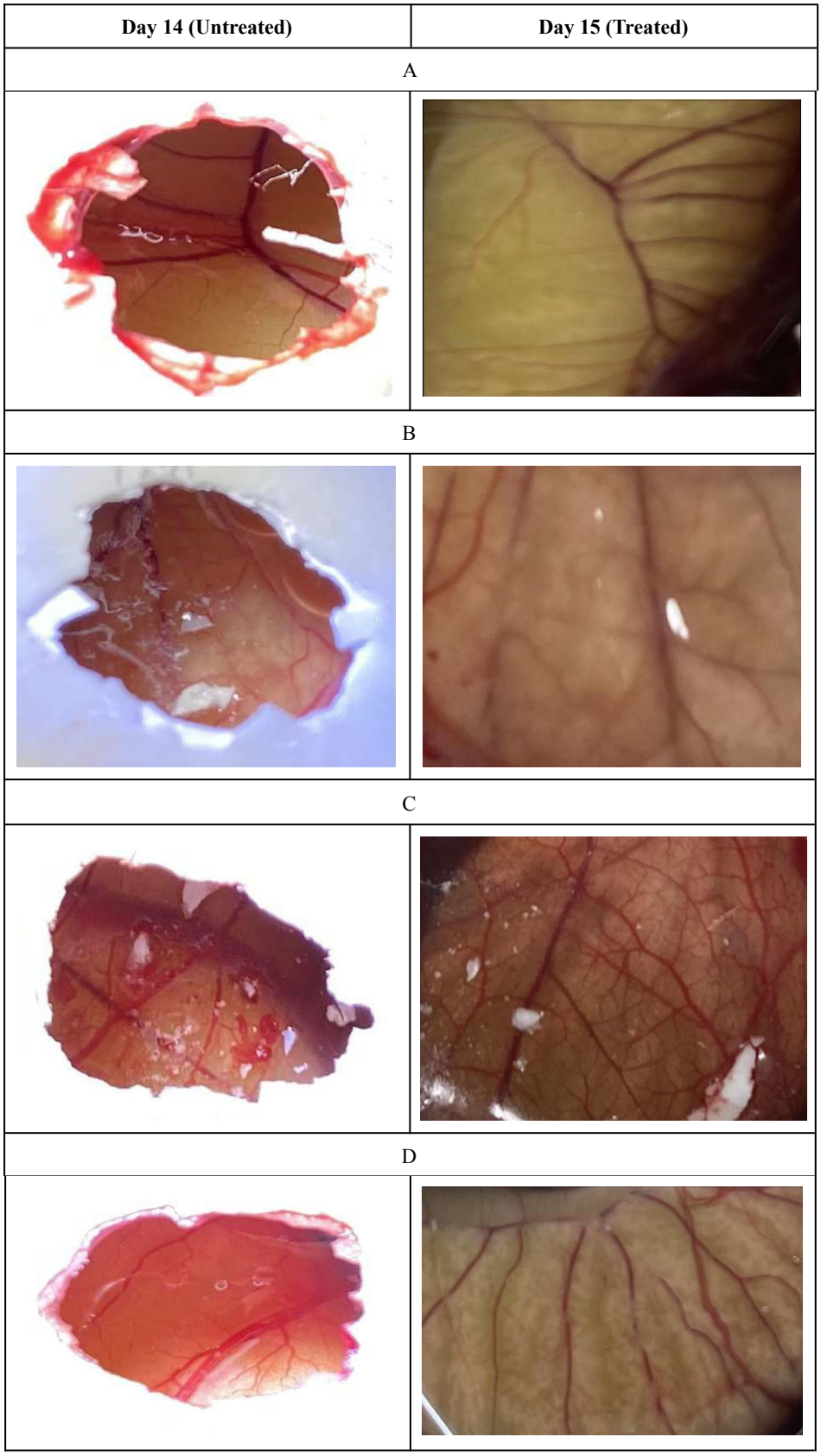
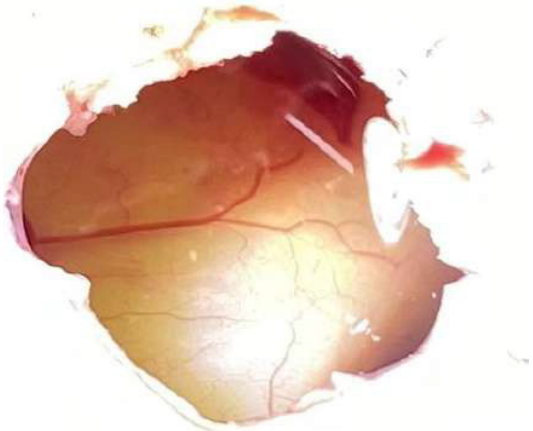
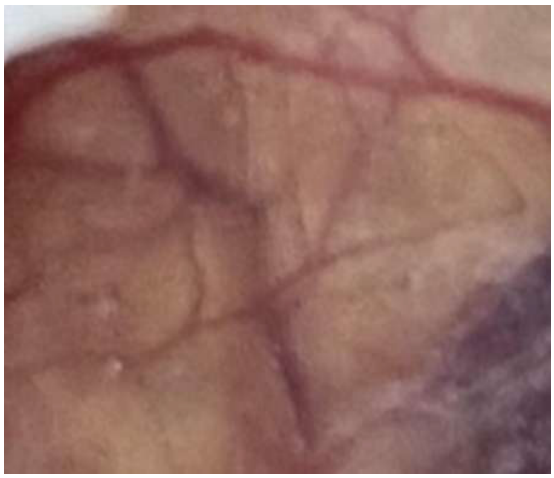
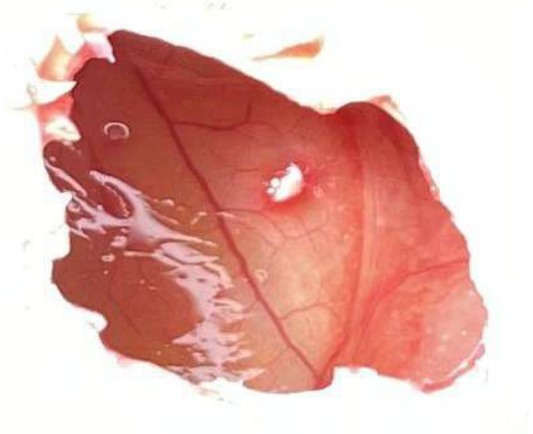
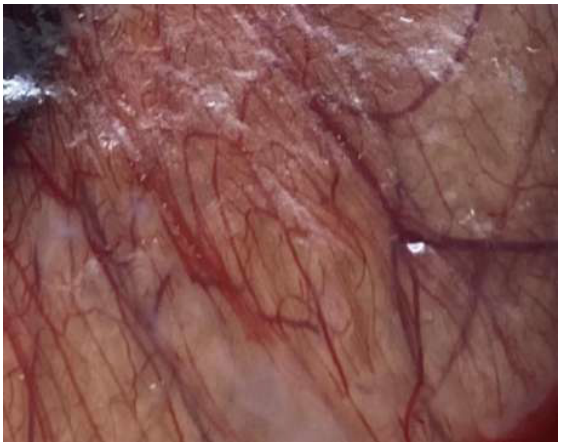


Figure 2: CAM assay treatments at 30µg/mL (A) Negative control (-43.45 ± 3.08% of inhibition); (B) CAM treated with *D. dao* (-279.56 ± 3.08% of inhibition); (C) CAM treated with *D. dao* (-279.56 ± 3.08% of inhibition); (D) CAM treated with *Z. talanai* (-136.55 ± 3.08% of inhibition)

At the 30.00µg/mL concentration, none of the plant extracts showed angiogenesis inhibition. According to the statistical analysis, there were no discernible differences between *D. dao, Z. talanai*, and the negative control.

Treatments	Trial 1	Trial 2	Trial 3	Mean	S.E.M.
Distilled water	-407.69%	-462.69%	0%	-290.13%	±4.18%
D. dao	10.26%	27.42%	0%	12.56%	±4.18%

Table 4: Summary of Percent Angiogenesis Inhibition At 50µg/ml with The Computed Mean and Standard Error of Mean

Day 14 (Untreated)	Day 15 (Treated)
A	
	
B	
	

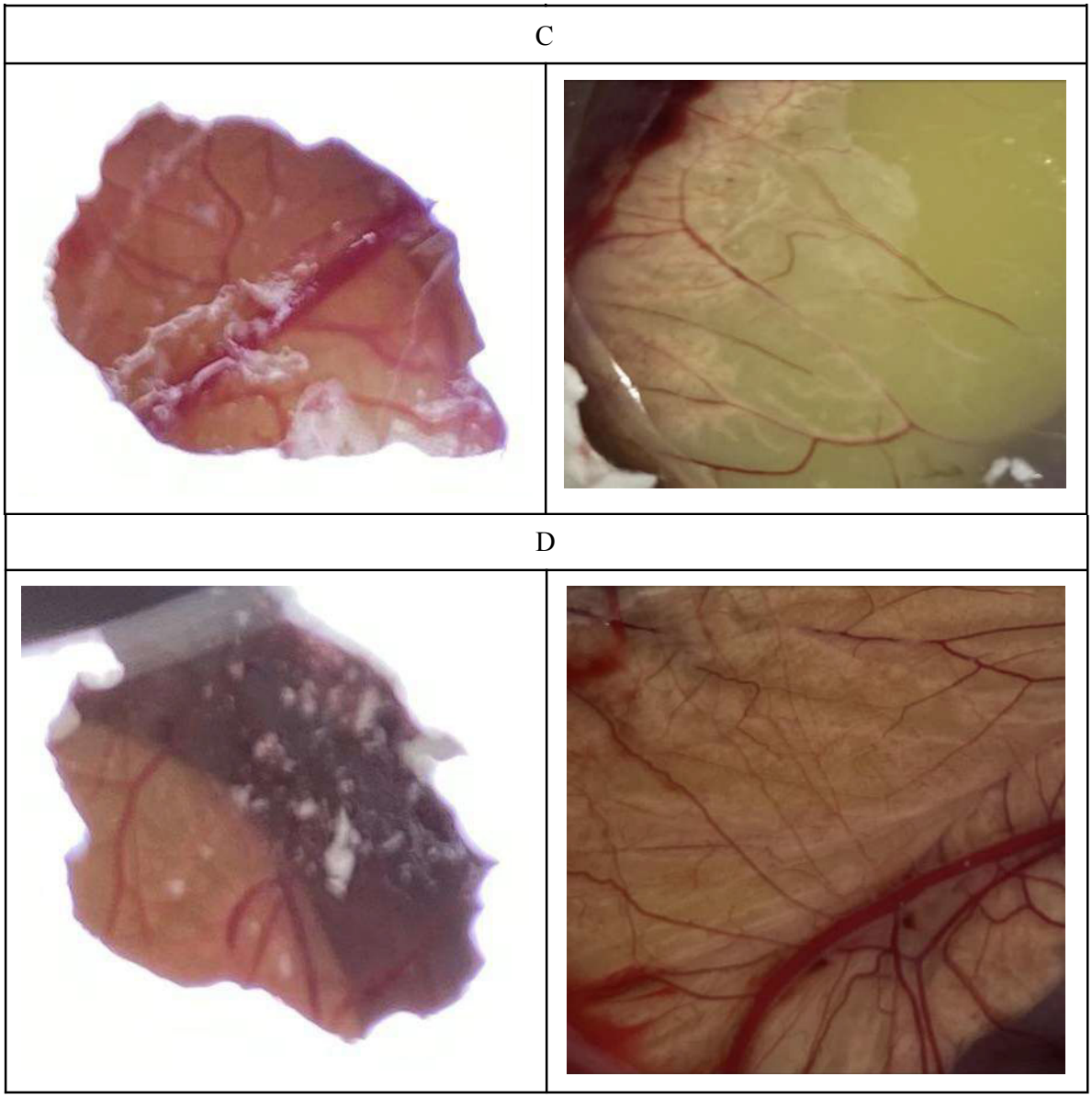
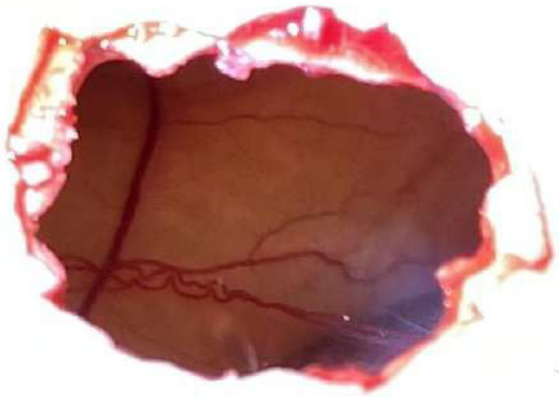
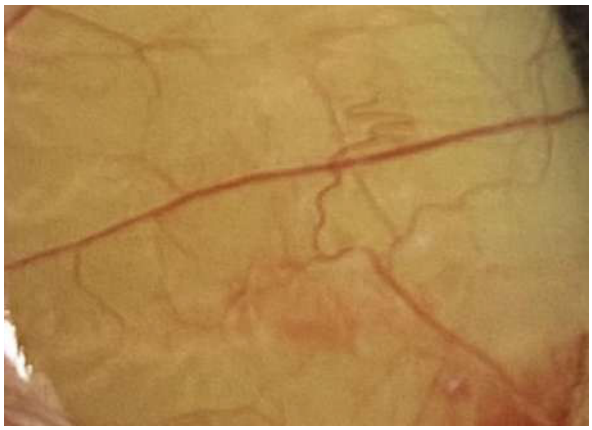
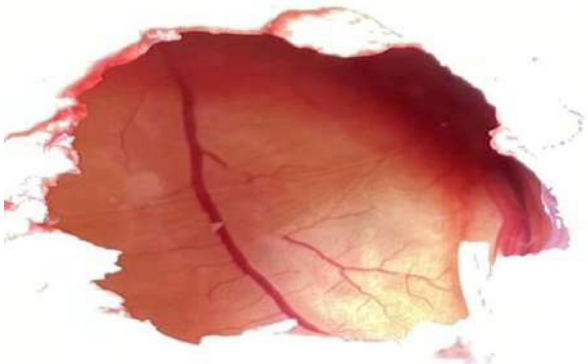
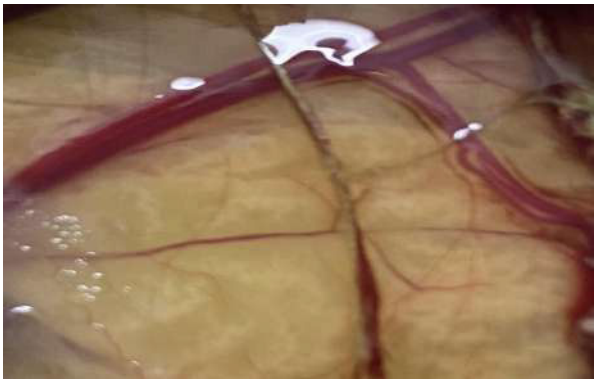


Figure 3: CAM assay treatments at 50µg/mL (A) Negative control (-290.13 ± 4.18% of inhibition); (B) Negative control (-290.13 ± 4.18% of inhibition); (C) CAM treated with *D. dao* (12.56 ± 4.18% of inhibition); (D) CAM treated with *D. dao* (12.56 ± 4.18% of inhibition)

For the 50µg/mL concentration, the *D. dao* treatment was the only one that survived out of all the treatment groups, including the negative control. According to the statistical analysis, *D. dao* showed discernible variations when compared to the negative control, with percent angiogenesis inhibitions of 12.56 ± 4.18% and -290.13 ± 4.18%, respectively.

Treatments	Trial 1	Trial 2	Trial 3	Mean	S.E.M.
Distilled water	5.44%	0%	0%	1.81%	±1.02%
Quercetin	74.19%	0%	0%	24.73%	±1.02%
D. dao	-12%	0%	0%	-4.00%	±1.02%

Table 5: Summary of Percent Angiogenesis Inhibition At 100µg/ml With the Computed Mean and Standard Error of Mean

Day 14 (Untreated)	Day 15 (Treated)
A	
	
B	
	

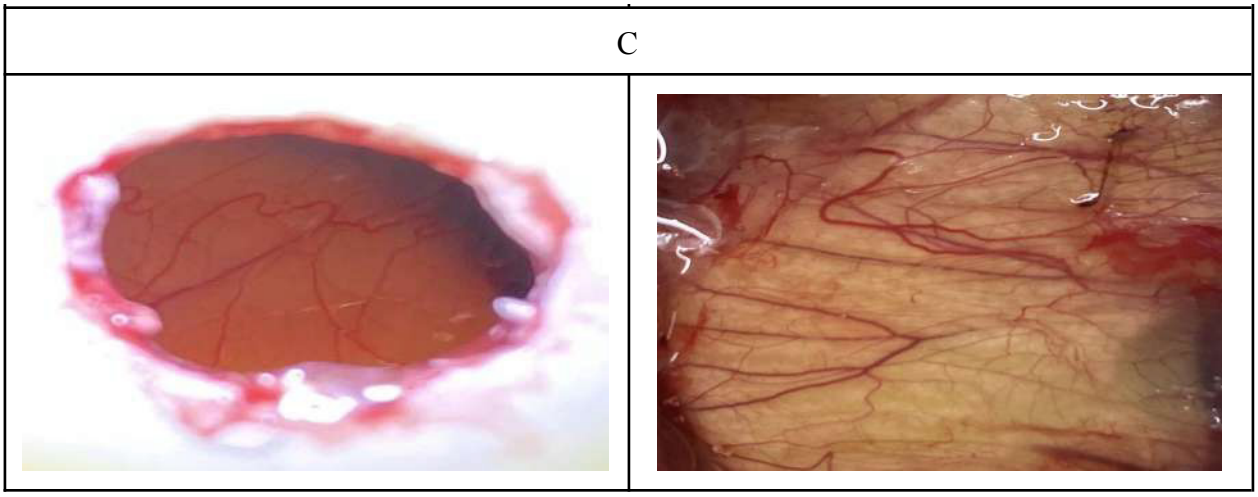


Figure 4: CAM assay treatments at 100µg/mL (A) Negative control (1.81 ± 1.02% of inhibition); (B) CAM treated with Quercetin (24.73 ± 1.02% of inhibition); (C) CAM treated with *D. dao* (-4.00 ± 1.02% of inhibition)

Similar to the 50µg/mL concentration, the *D. dao* treatment was the only one to survive at 100µg/mL, showing a -4.00 ± 1.02% inhibition. According to the statistical analysis, *D. dao* exhibited significant differences with the negative and positive control with percent inhibitions of 1.81 ± 1.02% and 24.73 ± 1.02% respectively.

4.3. Drabkin Reagent Test

As a confirmation test for the results of the CAM assay quantification, the Drabkin reagent test was conducted. The findings then revealed the absorbance in each CAM. The negative control was utilized as a reference point (100%) to assess how treatment groups affected the hemoglobin produced by the embryo's blood vessels.

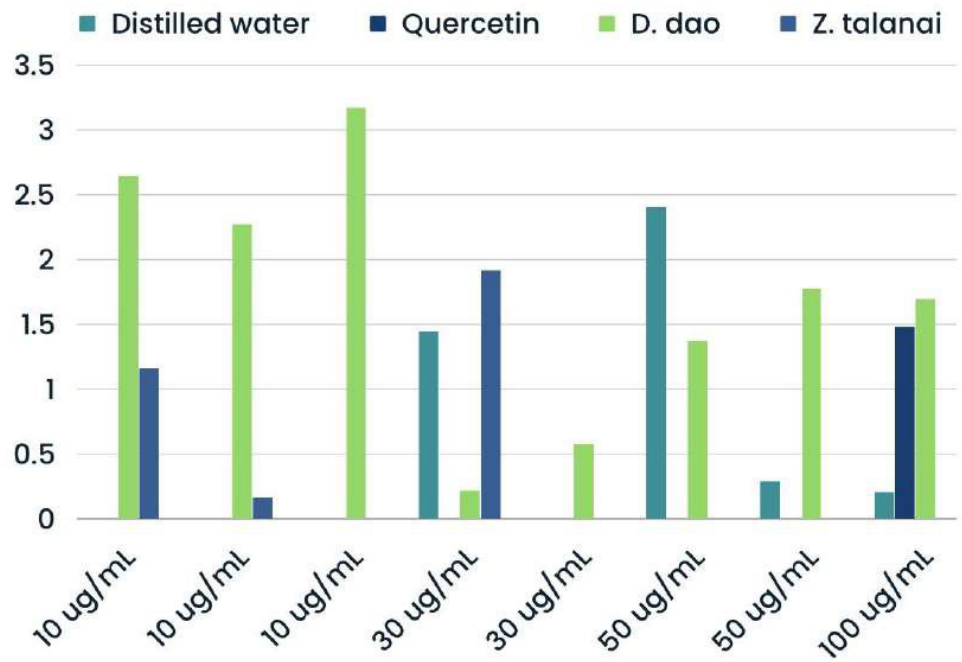


Figure 5: Results of the Drabkin reagent test

At a concentration of 10µg/mL, *D. dao* revealed 2.65%, 2.27%, and 3.17% hemoglobin content, while 1.16% and 0.17% of the hemoglobin content was shown in *Z. talanai*. While the hemoglobin concentration was present in the treatment groups with 30µg/mL, the findings showed 1.45% (Distilled water), 0.22% and 0.58% (*D. dao*), and 1.92% (*Z. talanai*). Hemoglobin concentrations for the 50µg/mL treatments were 1.37% and 1.78% for *D. dao*, and 2.41% and 0.29% for distilled water. Finally, the

amounts of hemoglobin for the 100µg/mL treatments were 0.21% for distilled water, 1.48% for Quercetin, and 1.69% for *D. dao*. The findings demonstrated that if the hemoglobin concentration is lower than the positive control, it means that the native plant has more antiangiogenic potential.

5. Conclusion

In conclusion, the study found that *Dracontomelon dao*, *Terminalia catappa*, and *Ziziphus talanai* contain the phytochemicals anthraquinones, alkaloids, and flavonoids. Additionally, neither of the plant extracts of *Dracontomelon dao*, *Terminalia catappa*, nor *Ziziphus talanai* have antiangiogenic properties based on the chorioallantoic membrane (CAM) assay.

D. dao and *Z. talanai* plant extracts showed insignificant angiogenesis inhibition at concentrations of 10µg/mL and 30.00µg/mL, respectively. No discernible differences were found between *D. dao* or *Z. talanai* compared to the negative control at 30.00µg/mL concentration. Meanwhile, the *D. dao* treatment was the only one to survive at 50µg/mL, showing significant differences compared to the negative control. Similarly, the *D. dao* treatment showed a -4.00 ± 1.02% inhibition at 100µg/mL, showing significant differences with the negative and positive control.

Lastly, neither *Dracontomelon dao*, *Terminalia catappa*, nor *Ziziphus talanai* have the best antiangiogenic potential based on the analysis of variance (ANOVA). Since only 16 eggs survived during the course of the study after treatment administration, it is suggested that there could be other factors that contributed to the rotting of 43 eggs such as the number of days of the duck eggs, incubation duration, humidity, and temperature.

Recommendations

To ensure the survival of duck eggs during the course of treatment, it is recommended to consider first the number of days of the duck eggs that you will purchase since it is important to note the amount of time that the eggs will be removed from the incubator. This is because once the humidity and temperature of the egg decreases, it could affect the development of the embryo. This is also the reason why 70% ethyl alcohol was used to clean the eggs instead of distilled water. In addition, since the duck eggs will be transported from the supplier to the laboratory, you also need to consider the stability of the blood vessels depending on the number of days because once the blood vessels are broken, the embryo will die and won't proceed in development. This is the reason why no rotation was made in the eggs during the course of incubation. It is also advised to purchase 13-day old eggs since its blood vessels are more stable and could withstand being out of the incubator for a few hours.

Second would be the incubation duration because there is a risk of contamination either in in ove or ex ove set ups due to the opening that was made in the shell. Hence, for the in ove setup, it is only advised to incubate the eggs for a maximum of 24 hours after the administration of treatments to avoid rotting of eggs. Third would be the humidity and temperature because as mentioned earlier, both should be stable in order to continue the embryo's development. In particular, the duck eggs need a constant humidity of 55 to 60 percent and a temperature of 37 degrees Celsius.

Moreover, the plants described above contain the phytochemicals anthraquinones, flavonoids, and alkaloids. Given their therapeutic qualities, it is advised to shed more light and safeguard these native plants since research on different natural chemical structures that impact tumor angiogenesis inhibition may lead to the creation of anti-cancer medications.

Conversely though, despite *T. catappa's* inclusion of flavonoids, alkaloids, and anthraquinones in the phytochemical analysis, its antiangiogenic activities were not observed. To further corroborate the encouraging findings, it is advised to test the aforementioned plant using another kind of antiangiogenic assay, such as gel plug and sponge implantation. It is also recommended to test other concentrations through toxicity tests and further isolation of the secondary metabolites through quantitative phytochemical screening to anticipate if the certain phytochemicals detected are the ones causing the angiogenesis suppression.

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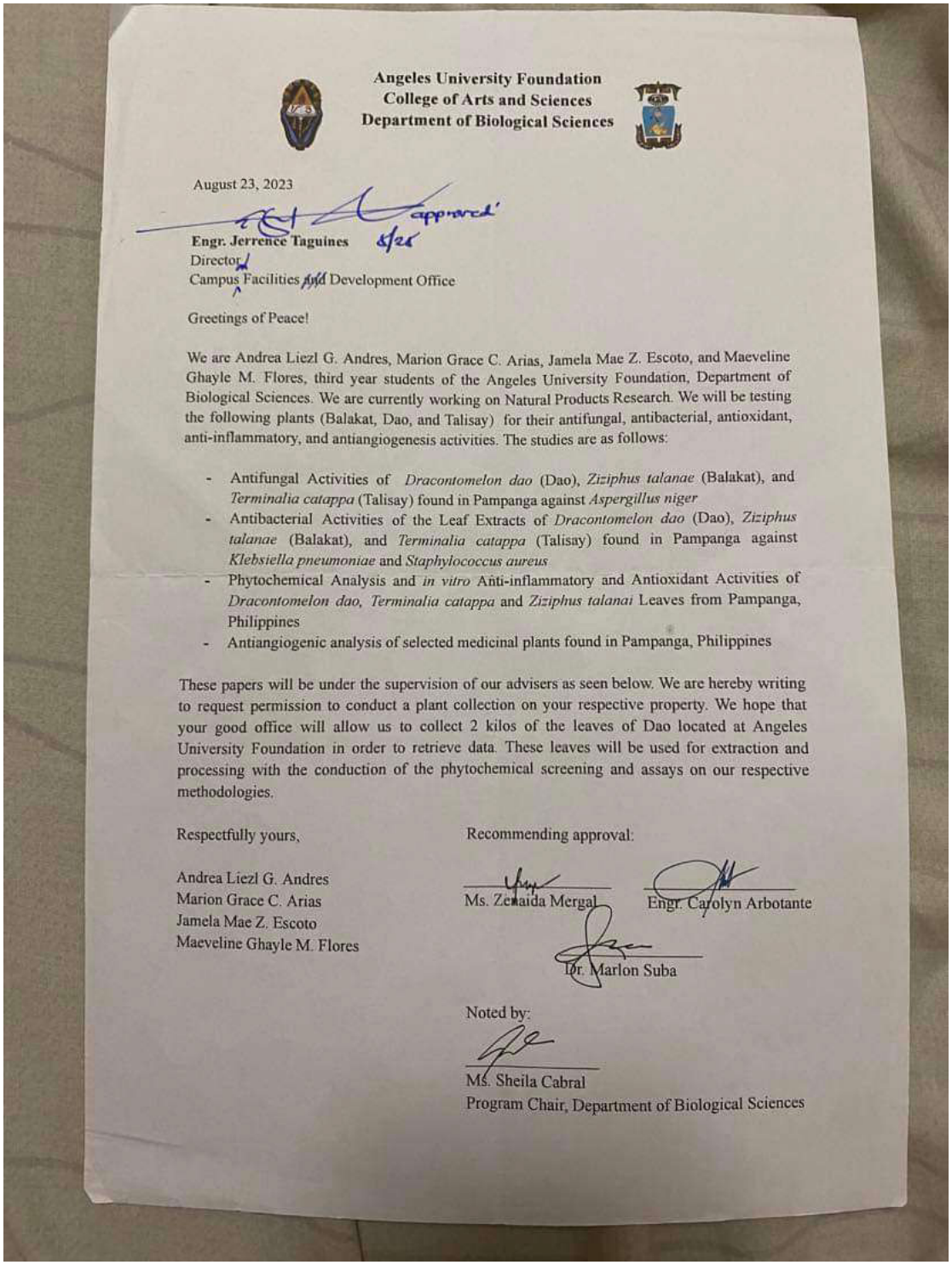
APPENDICES
Appendix A



Angeles University Foundation
College of Arts and Sciences
Department of Biological Sciences

August 23, 2023

Approved
8/24

Engr. Jerrence Taguines
Director
Campus Facilities and Development Office

Greetings of Peace!

We are Andrea Liezl G. Andres, Marion Grace C. Arias, Jamela Mae Z. Escoto, and Maeveline Ghayle M. Flores, third year students of the Angeles University Foundation, Department of Biological Sciences. We are currently working on Natural Products Research. We will be testing the following plants (Balakat, Dao, and Talisay) for their antifungal, antibacterial, antioxidant, anti-inflammatory, and antiangiogenesis activities. The studies are as follows:

- Antifungal Activities of *Dracontomelon dao* (Dao), *Ziziphus talanae* (Balakat), and *Terminalia catappa* (Talisay) found in Pampanga against *Aspergillus niger*
- Antibacterial Activities of the Leaf Extracts of *Dracontomelon dao* (Dao), *Ziziphus talanae* (Balakat), and *Terminalia catappa* (Talisay) found in Pampanga against *Klebsiella pneumoniae* and *Staphylococcus aureus*
- Phytochemical Analysis and *in vitro* Anti-inflammatory and Antioxidant Activities of *Dracontomelon dao, Terminalia catappa* and *Ziziphus talanai* Leaves from Pampanga, Philippines
- Antiangiogenic analysis of selected medicinal plants found in Pampanga, Philippines

These papers will be under the supervision of our advisers as seen below. We are hereby writing to request permission to conduct a plant collection on your respective property. We hope that your good office will allow us to collect 2 kilos of the leaves of Dao located at Angeles University Foundation in order to retrieve data. These leaves will be used for extraction and processing with the conduction of the phytochemical screening and assays on our respective methodologies.

Respectfully yours,

Andrea Liezl G. Andres
Marion Grace C. Arias
Jamela Mae Z. Escoto
Maeveline Ghayle M. Flores

Recommending approval:

Ms. Zenaida Mergal

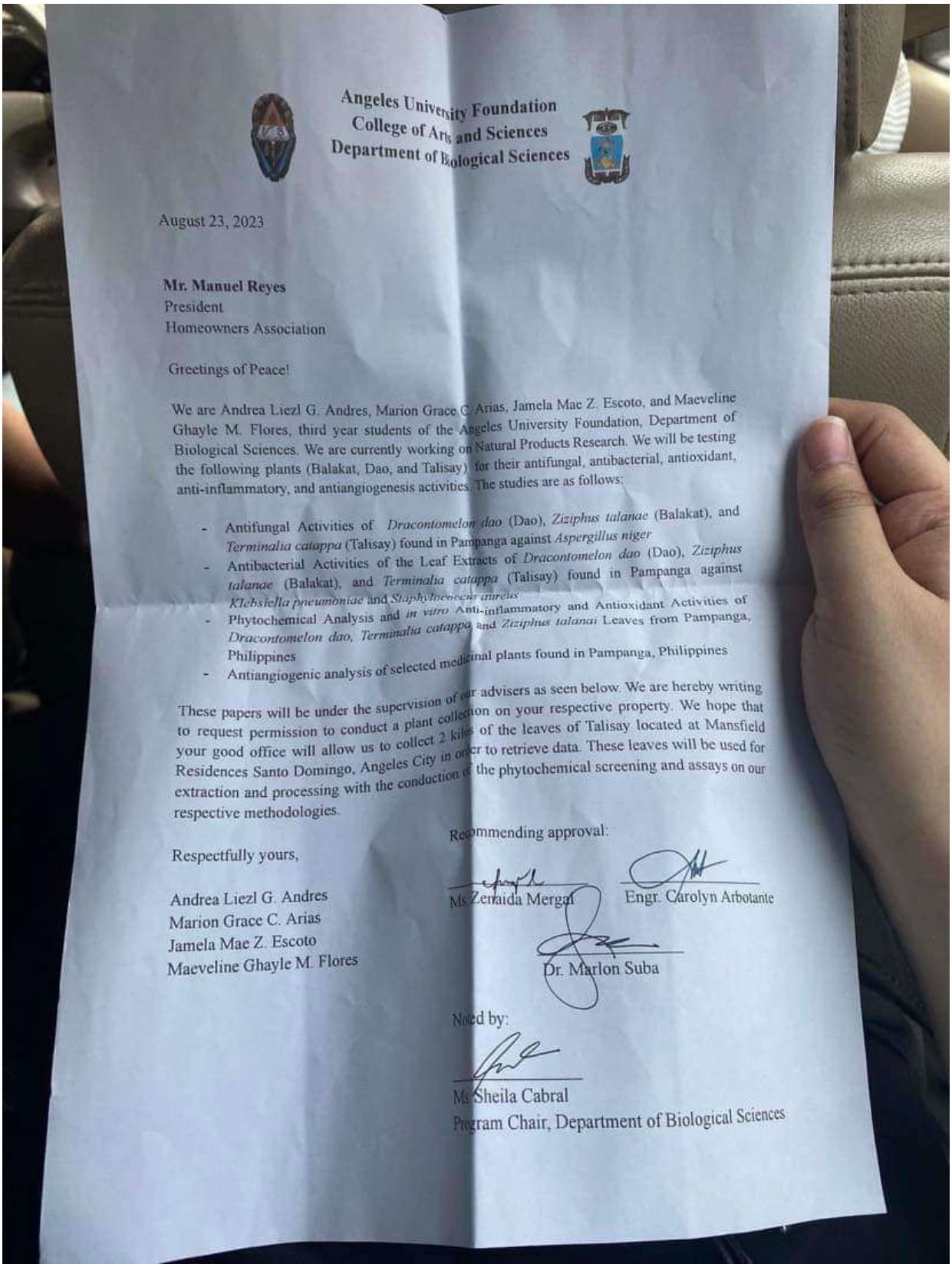
Engr. Carolyn Arbotante

Dr. Marlon Suba

Noted by:

Ms. Sheila Cabral
Program Chair, Department of Biological Sciences

Figure 1. Letter of permission for Dao plant collection in Angeles University Foundation.



Angeles University Foundation
College of Arts and Sciences
Department of Biological Sciences

August 23, 2023

Mr. Manuel Reyes
President
Homeowners Association

Greetings of Peace!

We are Andrea Liezl G. Andres, Marion Grace C. Arias, Jamela Mae Z. Escoto, and Maeveline Ghayle M. Flores, third year students of the Angeles University Foundation, Department of Biological Sciences. We are currently working on Natural Products Research. We will be testing the following plants (Balakat, Dao, and Talisay) for their antifungal, antibacterial, antioxidant, anti-inflammatory, and antiangiogenesis activities. The studies are as follows:

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- Antibacterial Activities of the Leaf Extracts of *Dracontomelon dao* (Dao), *Ziziphus talanae* (Balakat), and *Terminalia catappa* (Talisay) found in Pampanga against *Klebsiella pneumoniae* and *Staphylococcus aureus*
- Phytochemical Analysis and *in vitro* Anti-inflammatory and Antioxidant Activities of *Dracontomelon dao, Terminalia catappa* and *Ziziphus talanai* Leaves from Pampanga, Philippines
- Antiangiogenic analysis of selected medicinal plants found in Pampanga, Philippines

These papers will be under the supervision of our advisers as seen below. We are hereby writing to request permission to conduct a plant collection on your respective property. We hope that your good office will allow us to collect 2 kilos of the leaves of Talisay located at Mansfield Residences Santo Domingo, Angeles City in order to retrieve data. These leaves will be used for extraction and processing with the conduction of the phytochemical screening and assays on our respective methodologies.

Respectfully yours,

Andrea Liezl G. Andres
Marion Grace C. Arias
Jamela Mae Z. Escoto
Maeveline Ghayle M. Flores

Recommending approval:

Ms. Zenaida Mergal

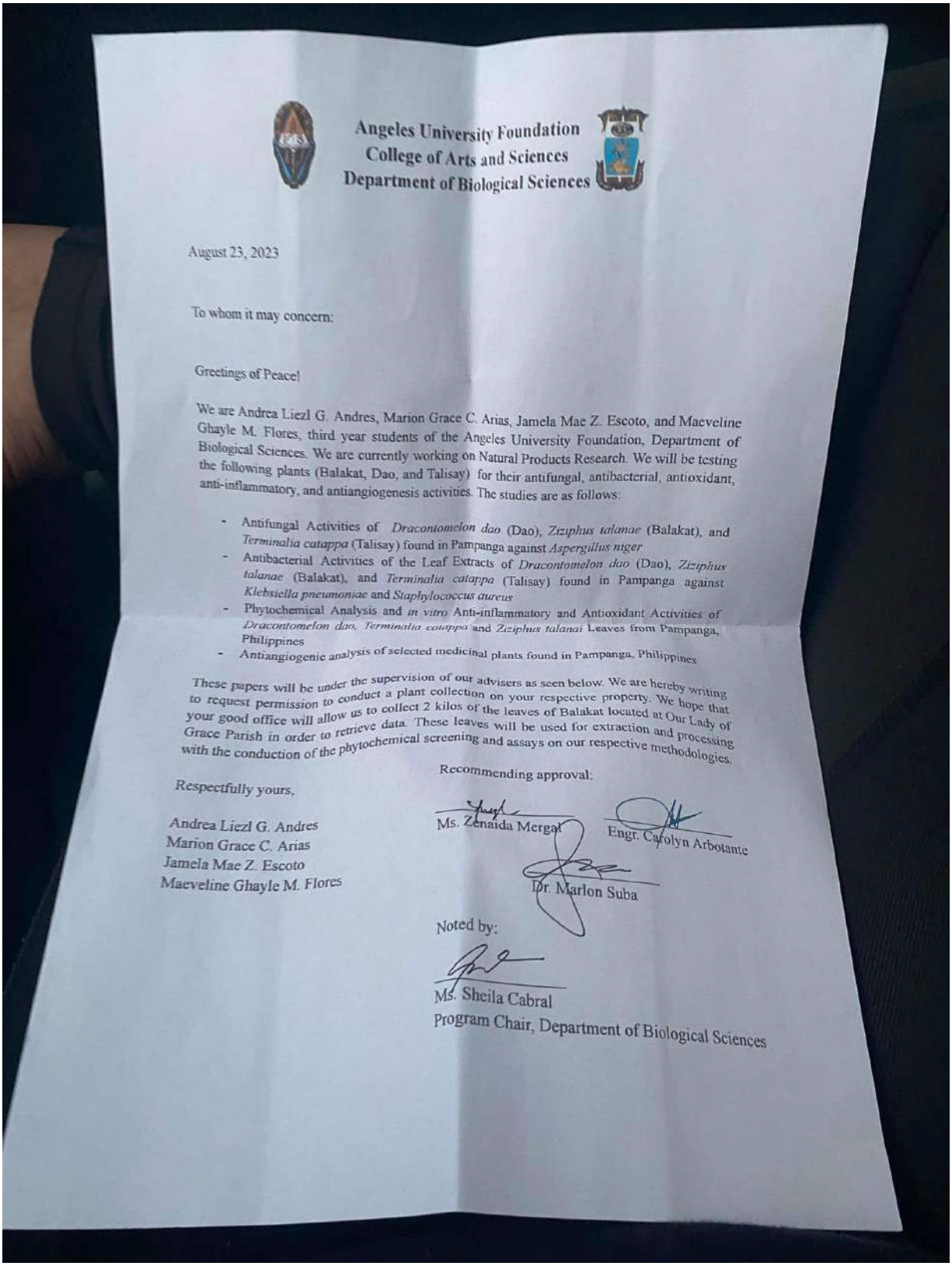
Engr. Carolyn Arbotante

Dr. Marlon Suba

Noted by:

Ms. Sheila Cabral
Program Chair, Department of Biological Sciences

Figure 2. Letter of permission for Talisay plant collection in Mansfield Residences.



Angeles University Foundation
College of Arts and Sciences
Department of Biological Sciences

August 23, 2023

To whom it may concern:

Greetings of Peace!

We are Andrea Liezl G. Andres, Marion Grace C. Arias, Jamela Mae Z. Escoto, and Maeveline Ghayle M. Flores, third year students of the Angeles University Foundation, Department of Biological Sciences. We are currently working on Natural Products Research. We will be testing the following plants (Balakat, Dao, and Talisay) for their antifungal, antibacterial, antioxidant, anti-inflammatory, and antiangiogenesis activities. The studies are as follows:

- Antifungal Activities of *Dracontomelon dao* (Dao), *Ziziphus talanae* (Balakat), and *Terminalia catappa* (Talisay) found in Pampanga against *Aspergillus niger*
- Antibacterial Activities of the Leaf Extracts of *Dracontomelon dao* (Dao), *Ziziphus talanae* (Balakat), and *Terminalia catappa* (Talisay) found in Pampanga against *Klebsiella pneumoniae* and *Staphylococcus aureus*
- Phytochemical Analysis and *in vitro* Anti-inflammatory and Antioxidant Activities of *Dracontomelon dao, Terminalia catappa* and *Ziziphus talanai* Leaves from Pampanga, Philippines
- Antiangiogenic analysis of selected medicinal plants found in Pampanga, Philippines

These papers will be under the supervision of our advisers as seen below. We are hereby writing to request permission to conduct a plant collection on your respective property. We hope that your good office will allow us to collect 2 kilos of the leaves of Balakat located at Our Lady of Grace Parish in order to retrieve data. These leaves will be used for extraction and processing with the conduction of the phytochemical screening and assays on our respective methodologies.

Respectfully yours,

Andrea Liezl G. Andres
Marion Grace C. Arias
Jamela Mae Z. Escoto
Maeveline Ghayle M. Flores

Recommending approval:

Ms. Zenaida Mergal

Engr. Carolyn Arbotante

Dr. Marlon Suba

Noted by:

Ms. Sheila Cabral
Program Chair, Department of Biological Sciences

Figure 3. Letter of permission for Balakat plant collection in Our Lady of Grace Parish.

ANGELES UNIVERSITY FOUNDATION

COLLEGE OF ARTS AND SCIENCES

Department of Biological Sciences

October 12, 2023

OFFICE OF THE VP FOR RESEARCH AND INNOVATION (OVPRI)
REYNALDO D. L. BUNDALIAN JR., RMT, DrPH (MEDICAL MICROBIOLOGY)
VP for Research and Innovation
Email address: bundaliardjr.cas@auf.edu.ph

Dear Dr. Bundalian:

Greetings of Peace!

I'm Marion Grace C. Arias, a third year student from the Department of Biological Sciences in Angeles University Foundation. I am currently working on Natural Products Research wherein I will test the following plants (Balakat, Dao, and Talisay) for their antiangiogenic activities.

My paper entitled "*Antiangiogenic activities of selected medicinal plants found in Pampanga, Philippines*" will be under the supervision of my adviser as seen below. I am hereby writing to request for a UV spectrophotometer in order to measure the absorption of the Drabkin reagent and supernatant mixture for my confirmatory test. I hope that your good office will allow me to use the aforementioned instrument around November of this year in order to conduct my undergraduate research.

Respectfully yours,

Marion Grace C. Arias
BS Biology 3-B

Recommending approval:

_____
Dr. Marlon DL. Suba

Noted by:

_____
Ms. Sheila S. Cabral
Program Chair, Department of Biological Sciences

Figure 4. Letter of permission addressed to the OVPRI for UV spectrophotometer use.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
PAMPANGA STATE AGRICULTURAL UNIVERSITY
Magalang, Pampanga

NOTICE OF APPROVAL

Protocol No.: 033

Name of the Primary Investigator: **Marion Grace C. Arias**

Title of Proposed Study: **ANTIANGIOGENIC ACTIVITIES OF SELECTED MEDICINAL PLANTS FOUND IN PAMPANGA, PHILIPPINES**

Date of Application: **30 August 2023**

Date of ACU Protocol Review Assessment: 30 August 2023

The project proposal with **Protocol No. 033** is approved for implementation. The principal investigator is reminded to notify the committee once the project has started for IACUC to schedule visits for monitoring.

Approved:

ROGELIO P. CARANDANG JR., DVM, PhD
Member

MICHAEL ANGELO C. NICDAO, PhD
Member

CHERYL C. CABUNG, DVM
Member

NEIL C. TANQUILUT, DVM, PhDR
Chairman

Prepared by:

AILAINE JOY A. MARCOS
Secretary

Figure 5. Institutional Animal Care and Use Committee permit issued by Pampanga State Agricultural University

Appendix B

Table 1. Phytochemical screening guidelines from Guevarra (2005)

Phytochemical Screening Spray Reagents			
Phytoconstituents tested	**Test Spray Reagent**	**Treatment**	**Positive Result**
Flavonoids and steroids	Antimony (III) chloride	Spray and air dry	Glycosidic flavonoids: yellow to orange visible region; fluorescent color under UV light (365 nm)
Alkaloids	Dragendorff's reagent	Spray and air dry	Brown-orange spots emerge subsequent to spraying
Anthrones, anthraquinones, and coumarins	Methanolic KOH	Spray and inspect chromatogram under UV light (365 nm) and visible light	Coumarins - blue colored regions (UV -365 nm); Anthraquinones - orange color; Anthrones - yellow regions (UV - 365 nm)
Steroids and saponins	Acetic-anhydride sulfuric acid	Spray and inspect chromatogram under UV light (365 nm) and visible light	Fluorescence or dark spots

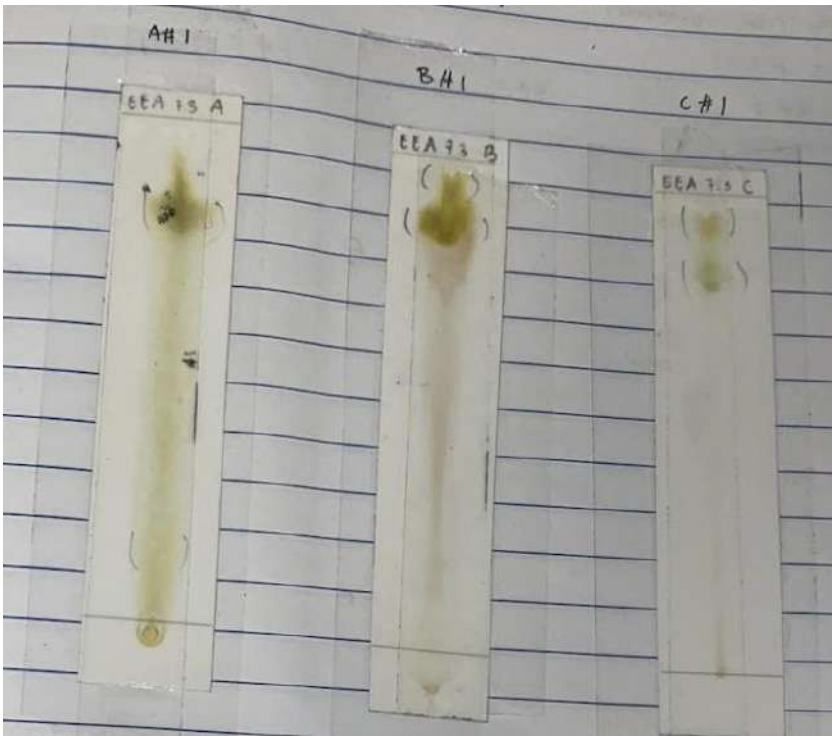


Figure 6. Phytochemical screening results of *D. dao*, *T. catappa*, and *Z. talanai* after spraying Antimony (III) chloride.

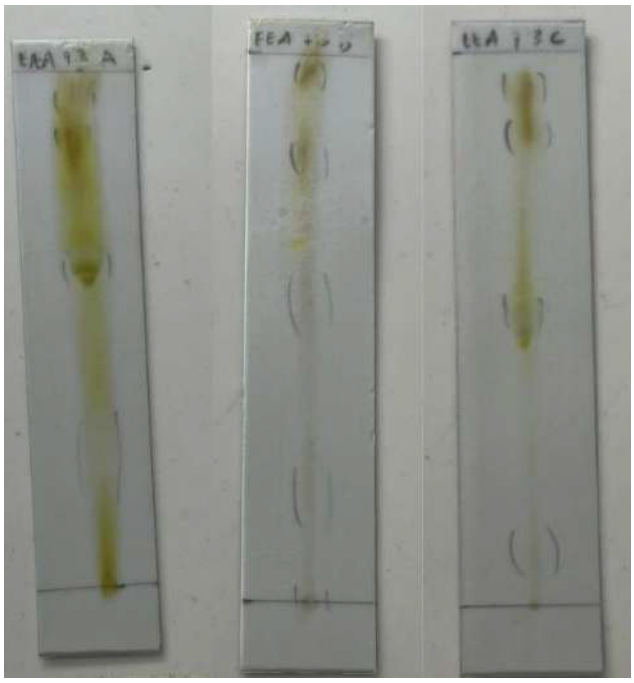


Figure 7. Phytochemical screening results of *D. dao*, *T. catappa*, and *Z. talanai* after spraying Dragendorff's reagent.

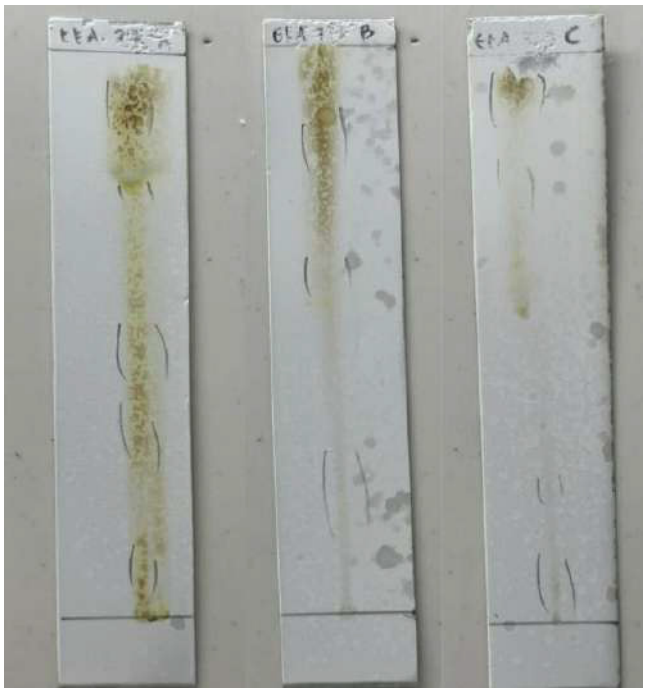


Figure 8. Phytochemical screening results of *D. dao*, *T. catappa*, and *Z. talanai* after spraying Methanolic KOH.

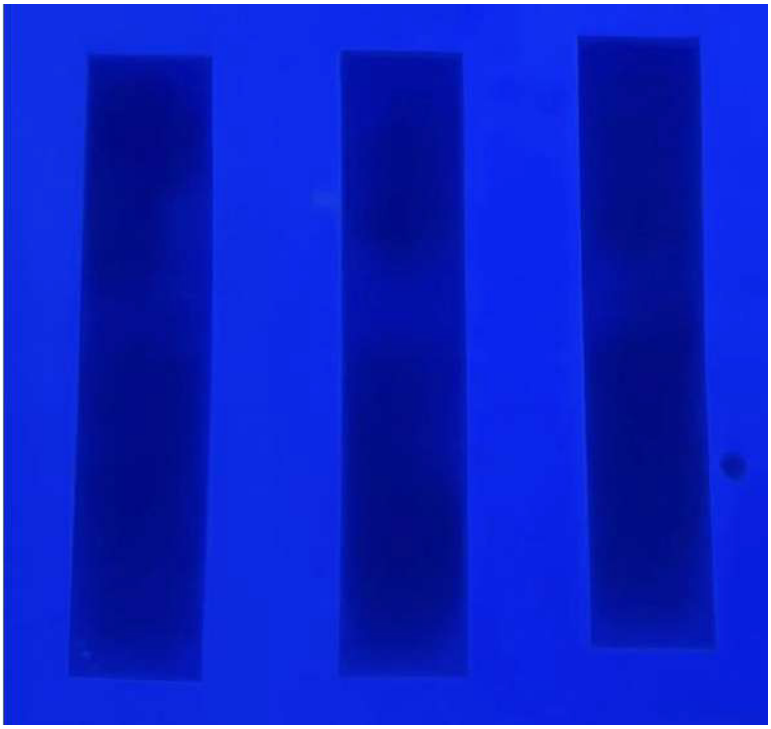


Figure 9. Phytochemical screening results of *D. dao*, *T. catappa*, and *Z. talanai* after spraying Acetic-anhydride sulfuric acid.

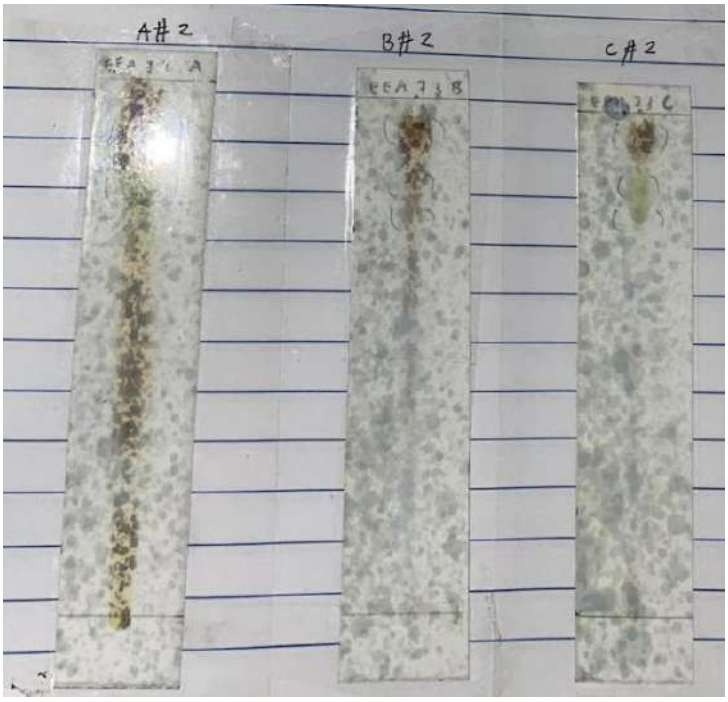


Figure 10. Phytochemical screening results of *D. dao*, *T. catappa*, and *Z. talanai* after spraying Potassium ferricyanide-ferric chloride.

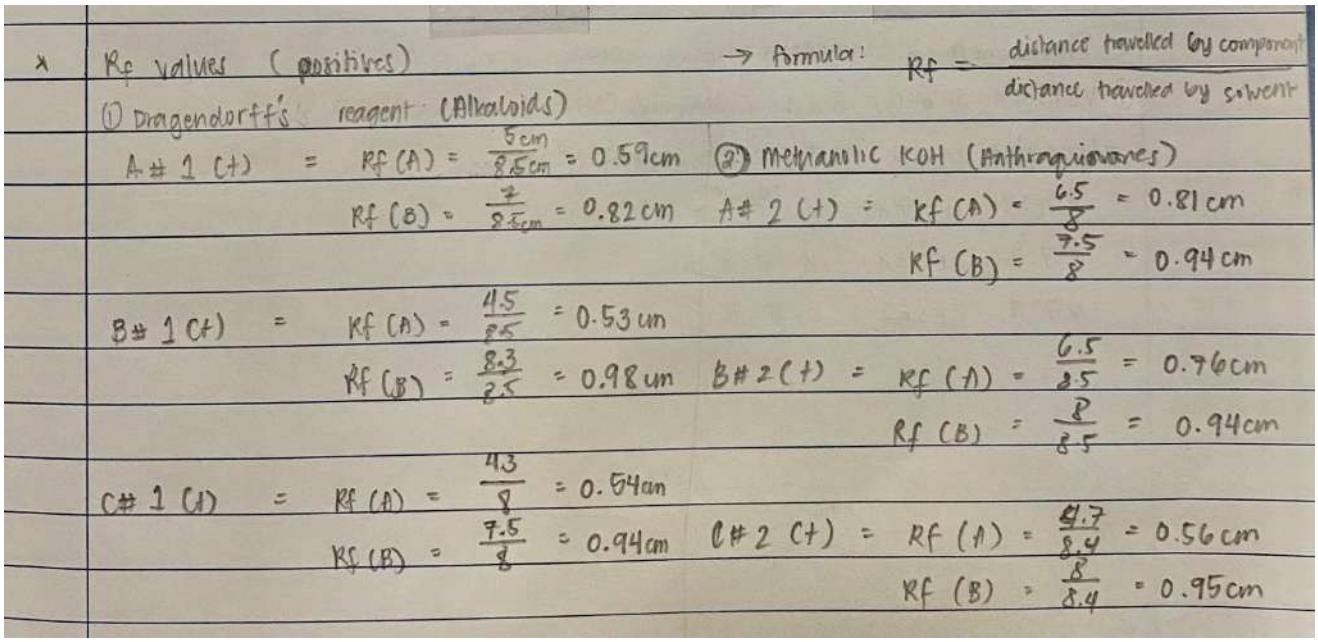


Figure 11. Rf values for Dragendorff's reagent and Methanolic KOH.

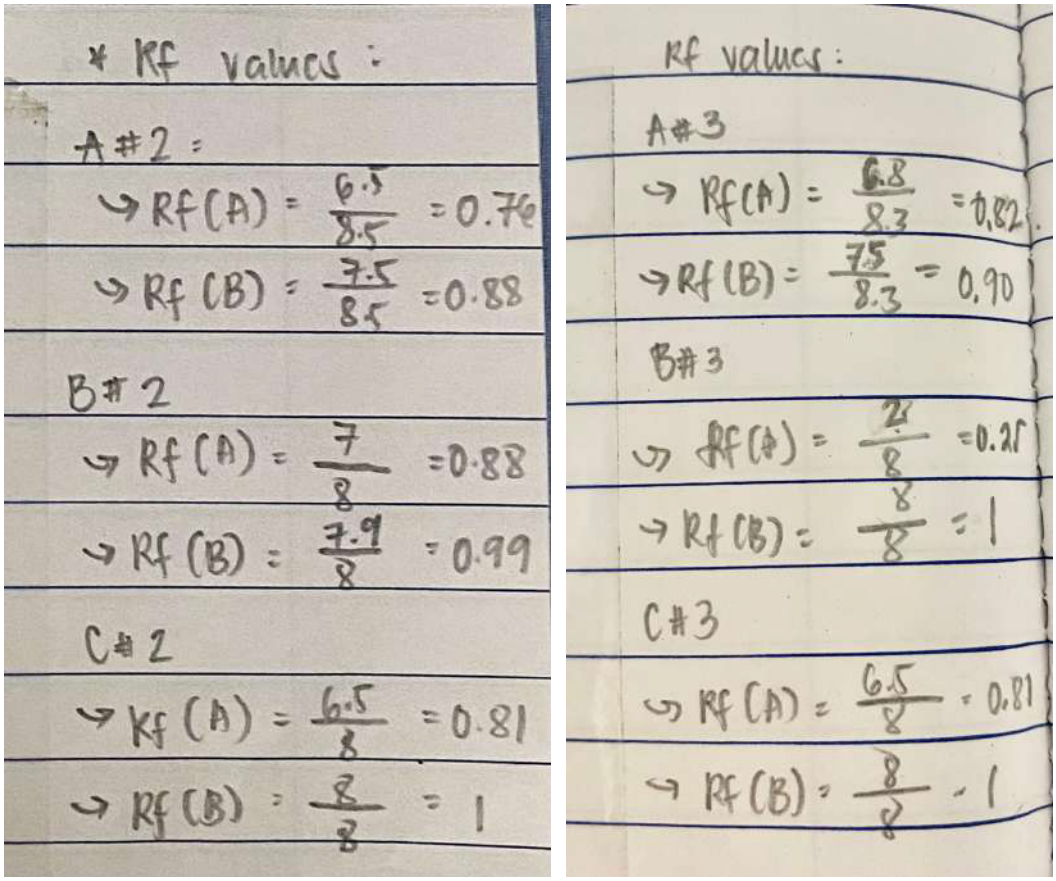


Figure 12. Rf values for Potassium ferricyanide-ferric chloride (left) and DPPH (right).

Appendix C

Table 2. Percentage of angiogenesis inhibition analysis from the treatments at 10µg/mL concentration.

Embryo code	Day 14	Day 15	% AIA
DA 1	211	507	-140.28
DA 2	126	336	-166.67
DA 3	94	271	-188.30
BA 1	253	252	0.40
BA 2	176	405	-130.11

*Treatments at 10µg/mL: DA 1-3 (*D. dao*); BA 1-2 (*Z. talanai*). These are the results generated by the ImageJ Angiogenesis Analyzer. The values under day 14 were the counted branches before administering the treatment while day 15 were the values 24 hours after treatment administration.

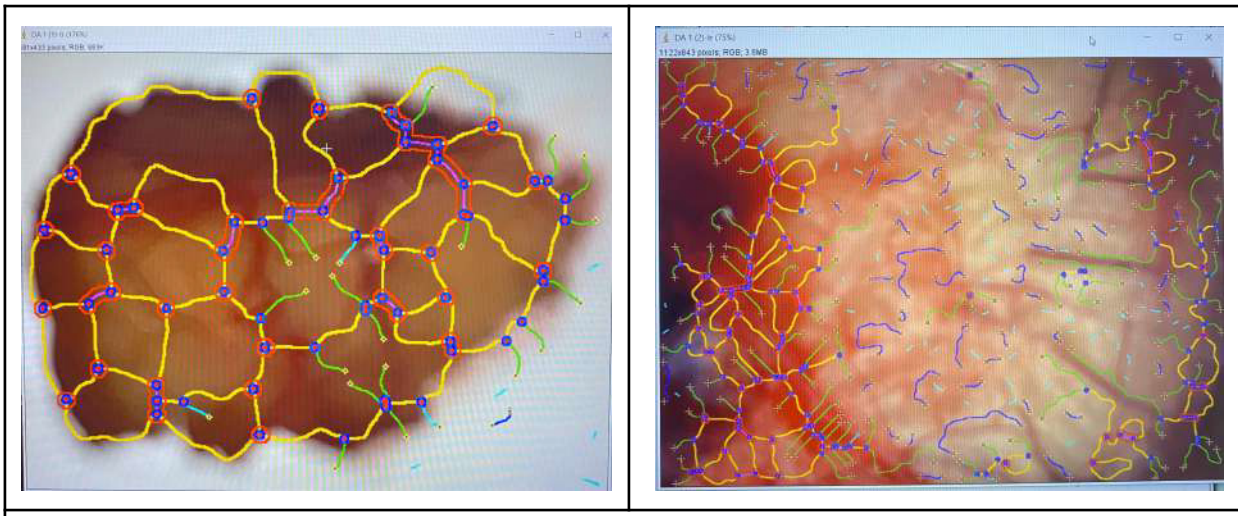


Figure 11. Images generated from ImageJ before (left) and after (right) the treatment of *D. dao* at 10µg/mL

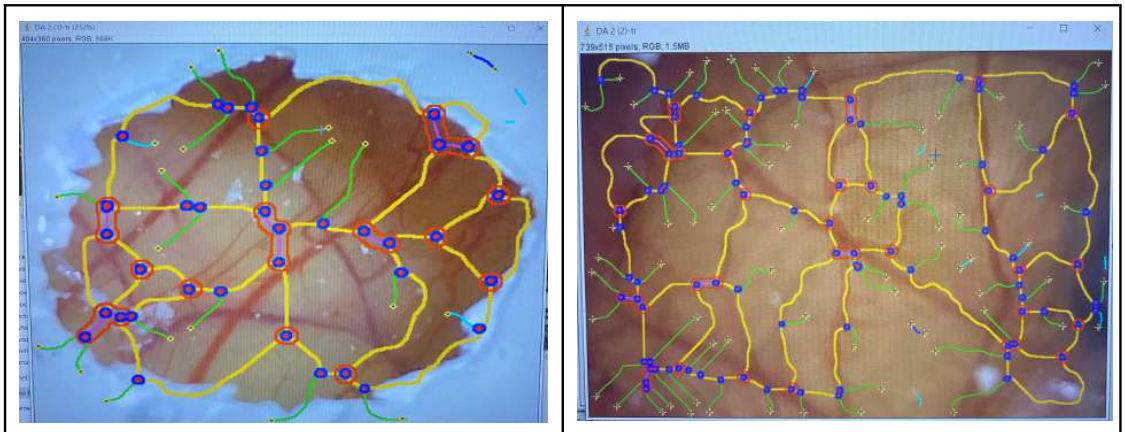


Figure 12. Images generated from ImageJ before (left) and after (right) the treatment of *D. dao* at 10µg/mL

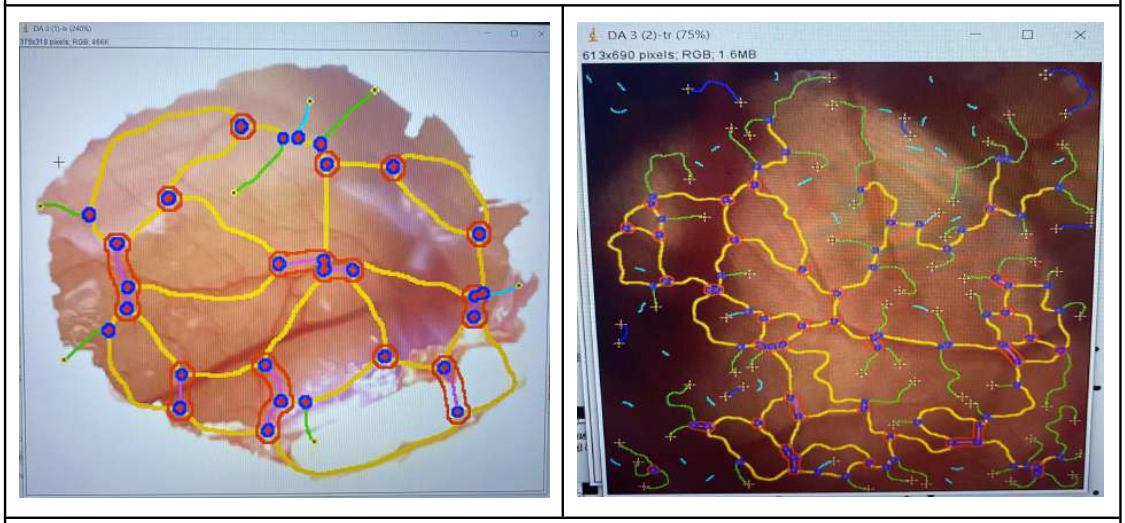


Figure 13. Images generated from ImageJ before (left) and after (right) the treatment of *D. dao* at 10µg/mL

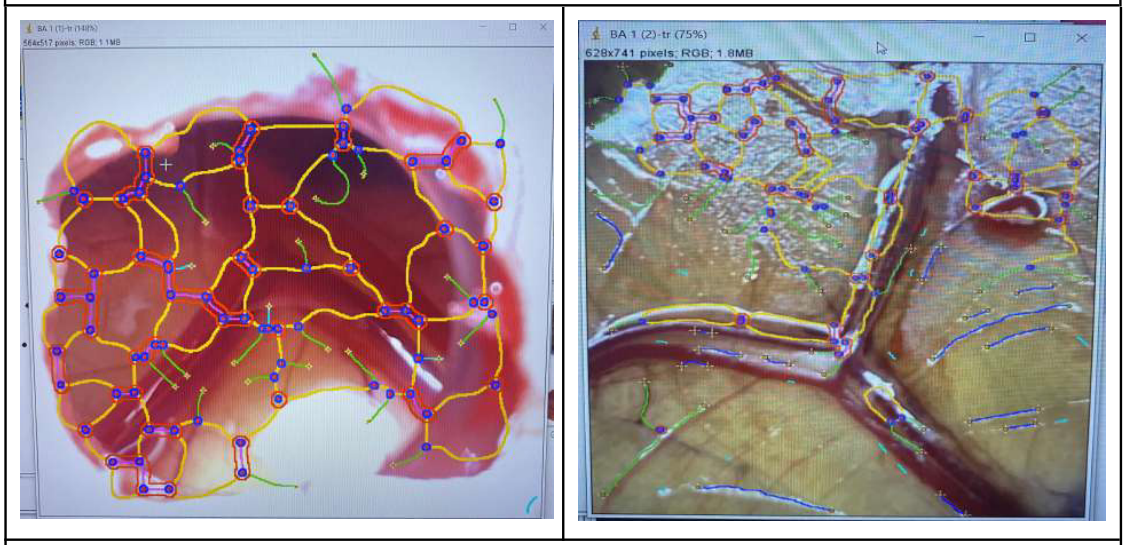


Figure 14. Images generated from ImageJ before (left) and after (right) the treatment of *Z. talanai* at 10µg/mL

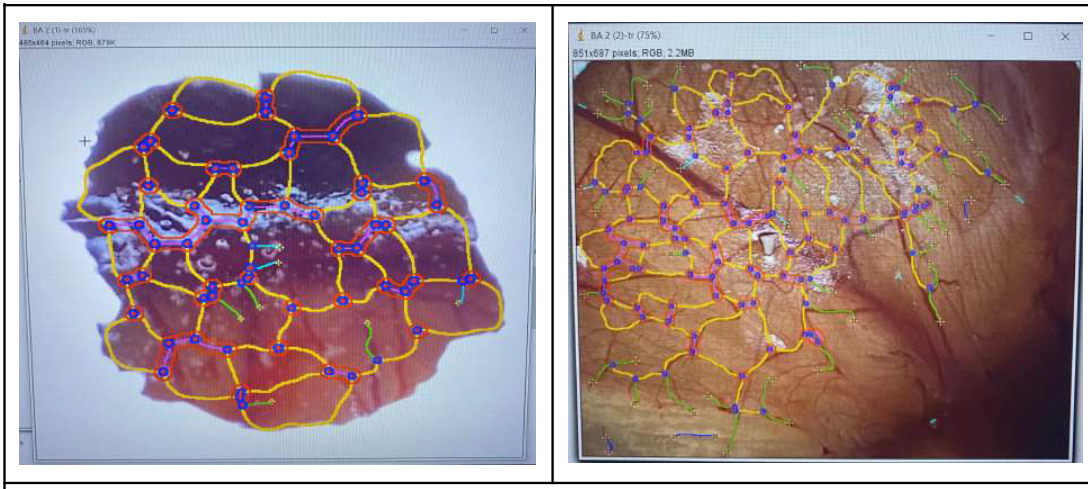


Figure 15. Images generated from ImageJ before (left) and after (right) the treatment of *Z. talanai* at 10µg/mL

Table 3. Percentage of angiogenesis inhibition analysis from the treatments at 30µg/mL concentration.

Embryo code	Day 14	Day 15	% AIA
- B 1	201	463	-130.35
DB 1	24	247	-929.17
DB 2	126	12	90.48
BB 1	114	581	-409.65

*Treatments at 30µg/mL: - B 1 (Negative control); DB 1-2 (*D. dao*); BB 1 (*Z. talanai*). These are the results generated by the ImageJ Angiogenesis Analyzer. The values under day 14 were the counted branches before administering the treatment while day 15 were the values 24 hours after treatment administration.

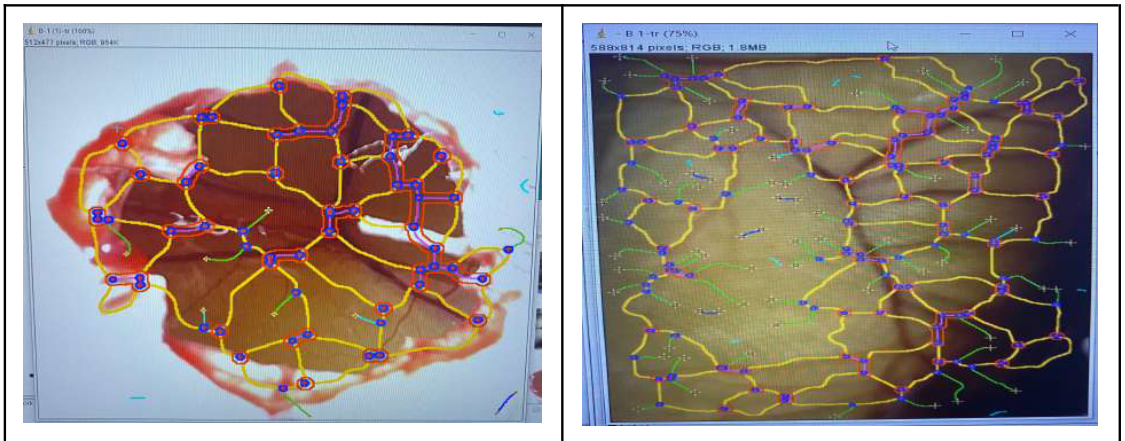


Figure 16. Images generated from ImageJ before (left) and after (right) the treatment of distilled water (negative control)

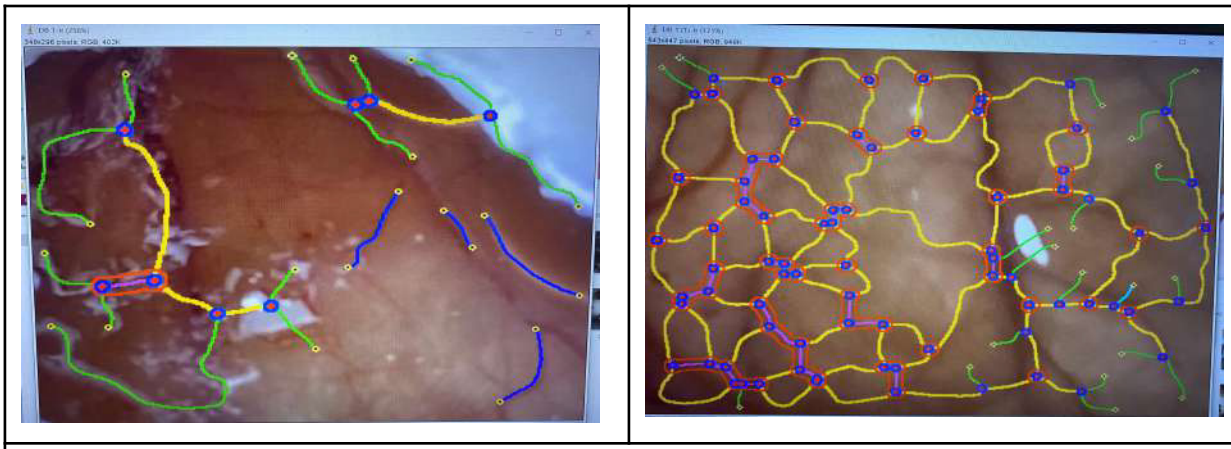


Figure 17. Images generated from ImageJ before (left) and after (right) the treatment of *D. dao* at 30µg/mL

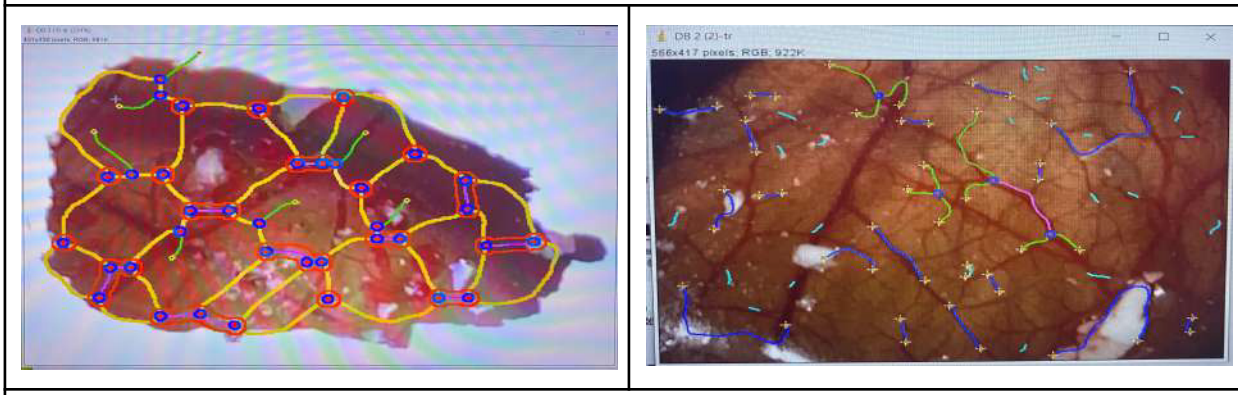


Figure 18. Images generated from ImageJ before (left) and after (right) the treatment of *D. dao* at 30µg/mL

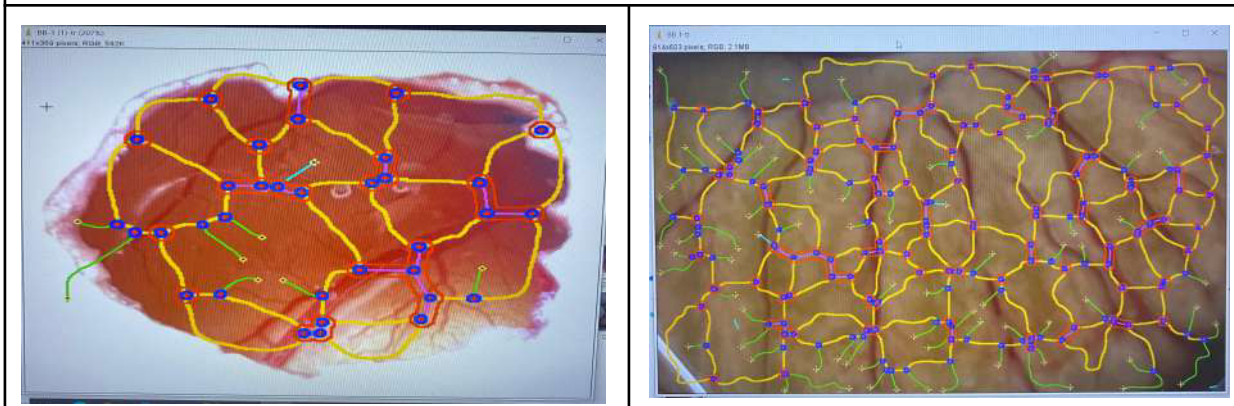


Figure 19. Images generated from ImageJ before (left) and after (right) the treatment of *Z. talanai* at 30µg/mL

Table 4. Percentage of angiogenesis inhibition analysis from the treatments at 50µg/mL concentration.

Embryo code	Day 14	Day 15	% AIA
- C 1	143	726	-407.69
- C 2	134	754	-462.69
DC 1	39	35	10.26
DC 2	186	135	27.42

*Treatments at 50µg/mL: - C 1-2 (Negative control); DC 1-2 (*D. dao*). These are the results generated by the ImageJ Angiogenesis Analyzer. The values under day 14 were the counted branches before administering the treatment while day 15 were the values 24 hours after treatment administration.

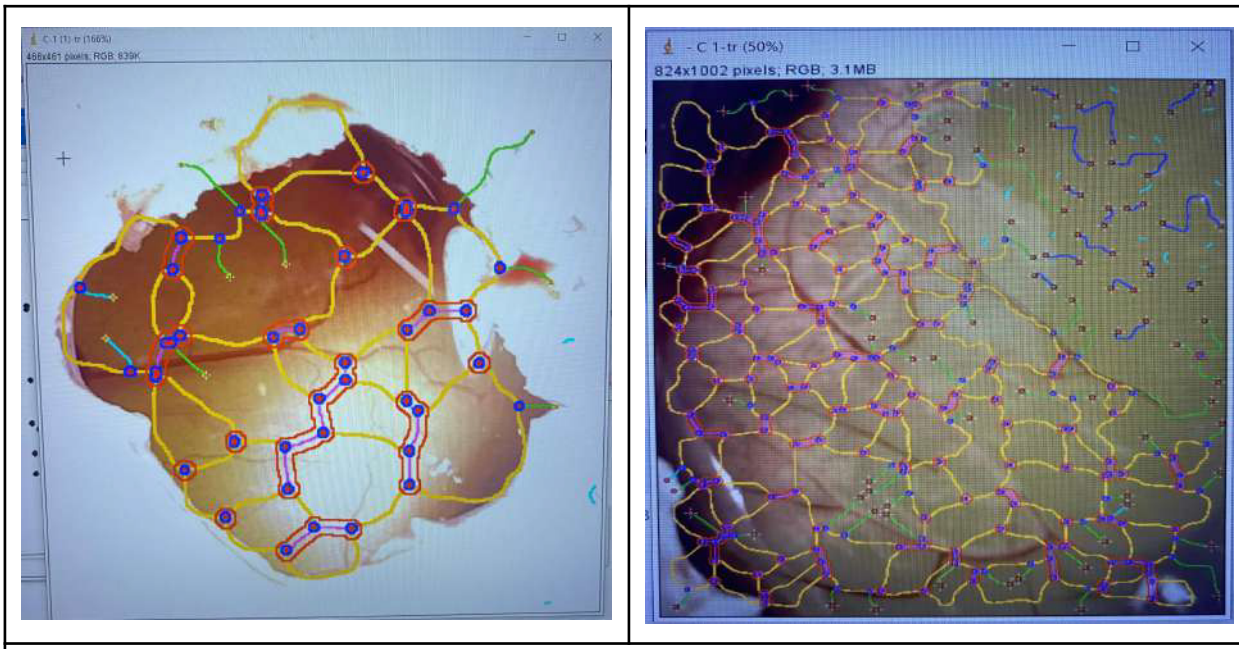


Figure 20. Images generated from ImageJ before (left) and after (right) the treatment of distilled water (negative control)

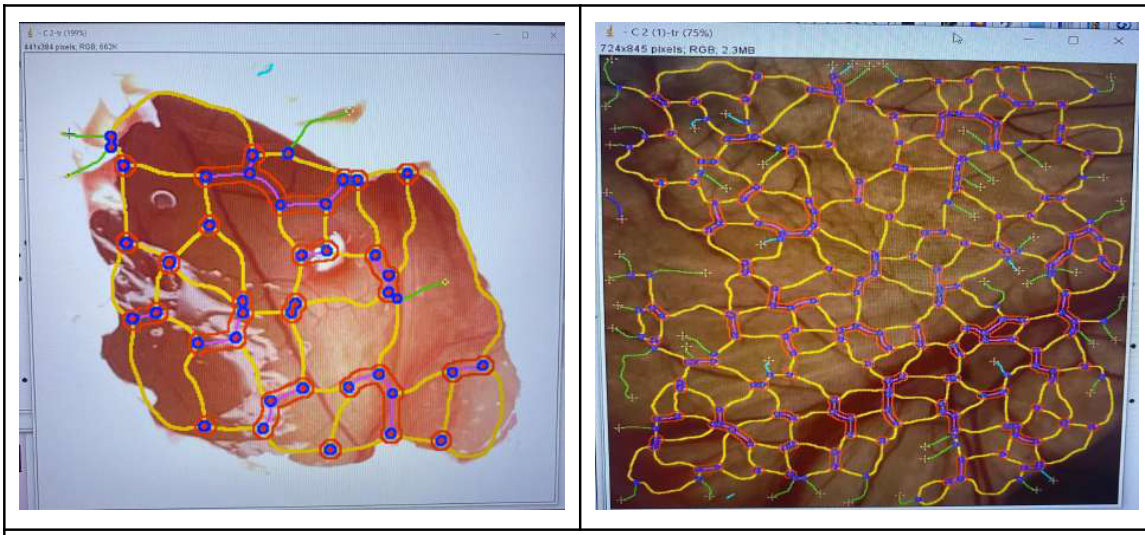


Figure 21. Images generated from ImageJ before (left) and after (right) the treatment of distilled water (negative control)

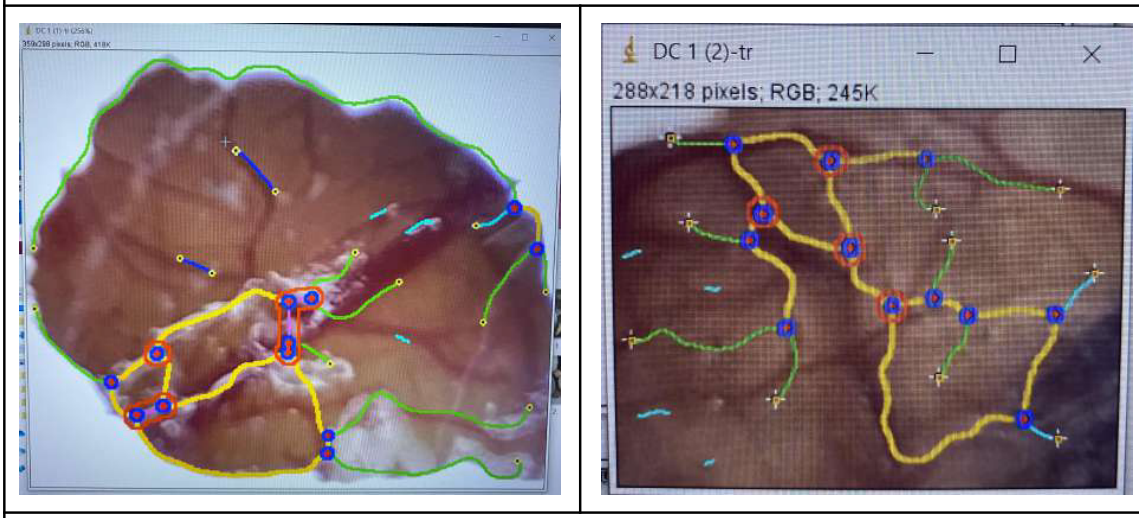


Figure 22. Images generated from ImageJ before (left) and after (right) the treatment of *D. dao* at 50µg/mL

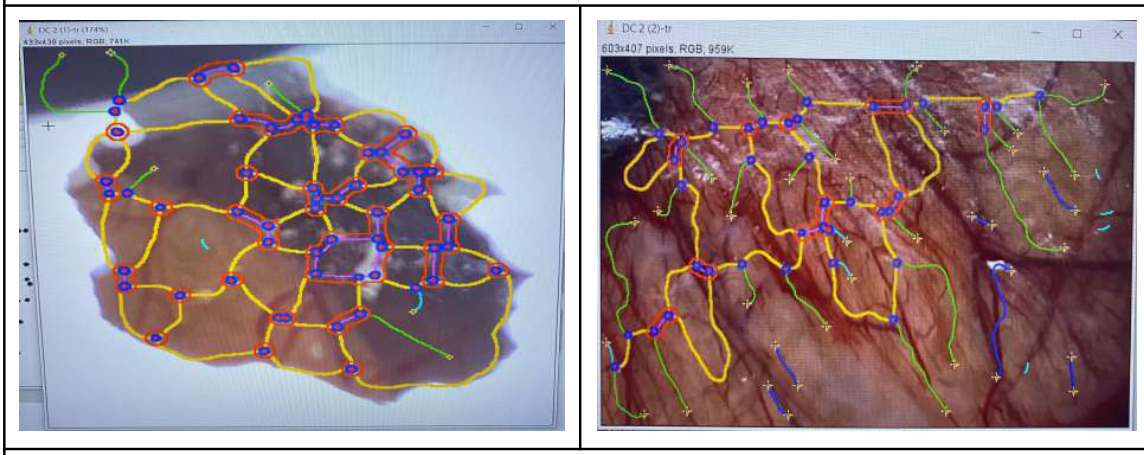


Figure 23. Images generated from ImageJ before (left) and after (right) the treatment of *D. dao* at 50µg/mL

Table 5. Percentage of angiogenesis inhibition analysis from the treatments at 100µg/mL concentration.

Embryo code	Day 14	Day 15	% AIA
- D 1	147	139	5.44
+ D 1	186	48	74.19
DD 1	125	140	-12.00

*Treatments at 100µg/mL: - D 1 (Negative control); + D 1 (Positive control); DD 1 (*D. dao*). These are the results generated by the ImageJ Angiogenesis Analyzer. The values under day 14 were the counted branches before administering the treatment while day 15 were the values 24 hours after treatment administration.

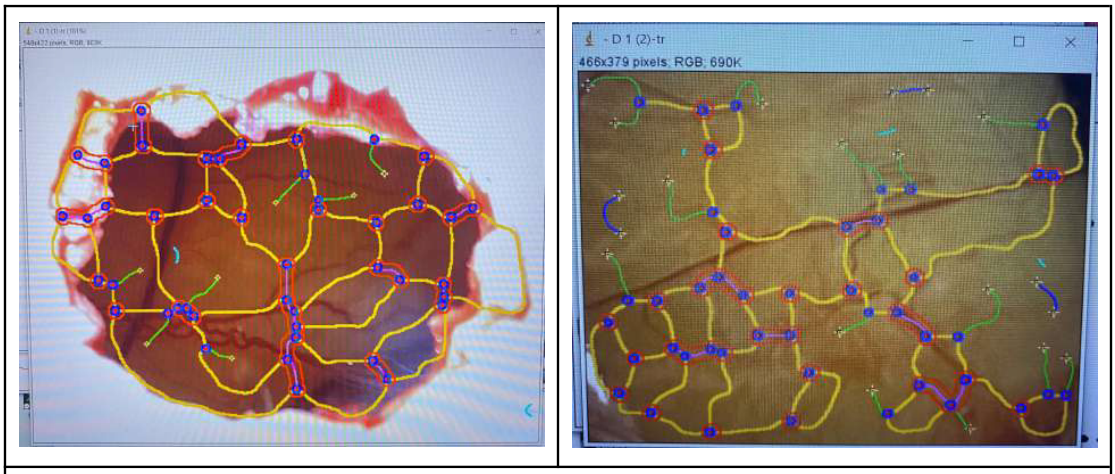


Figure 24. Images generated from ImageJ before (left) and after (right) the treatment of distilled water (negative control)

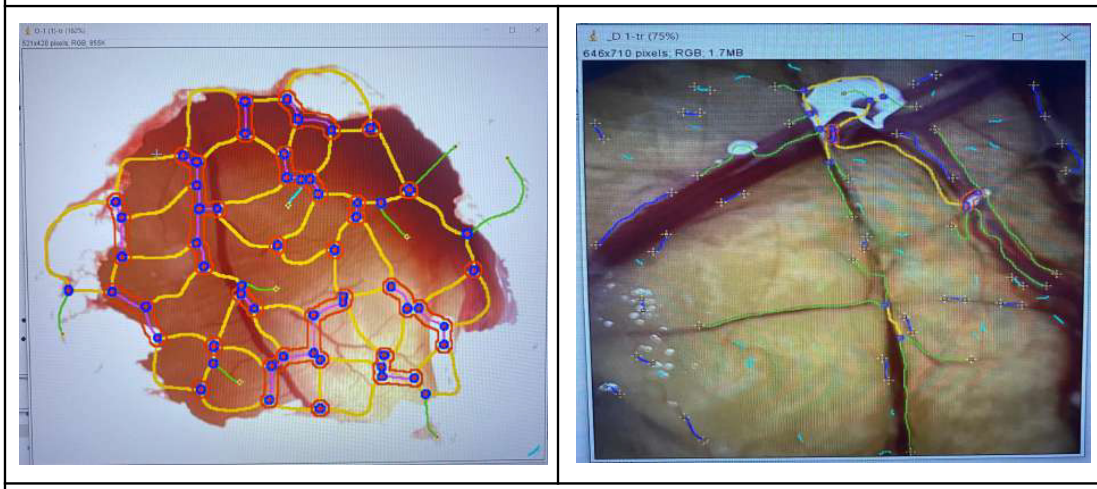


Figure 25. Images generated from ImageJ before (left) and after (right) the treatment of quercetin (positive control) at 100µg/mL

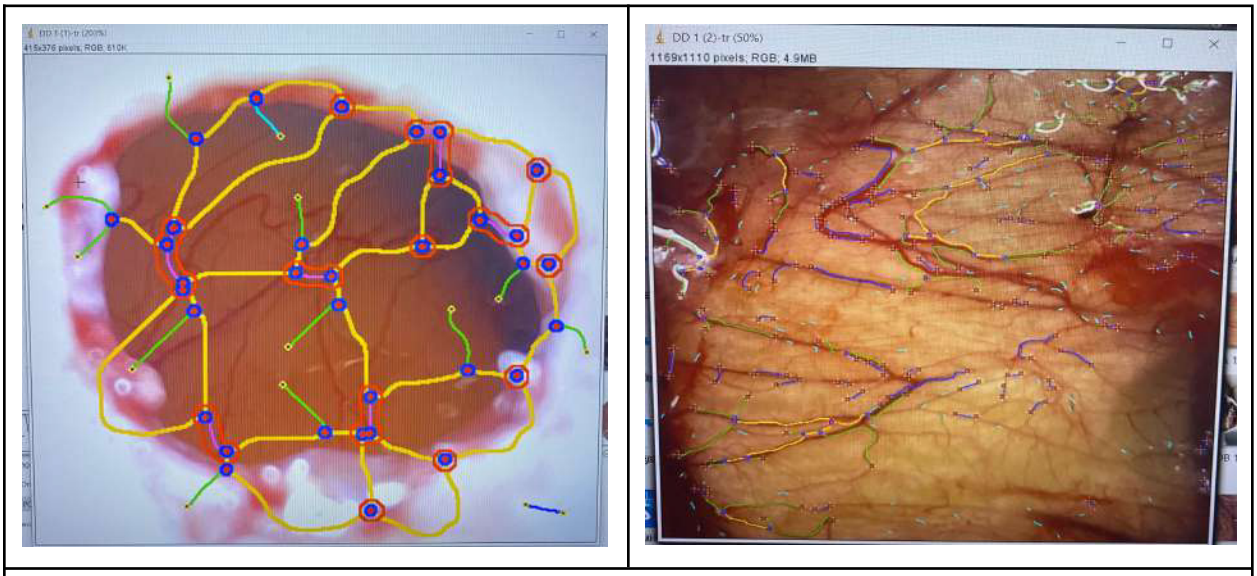


Figure 26. Images generated from ImageJ before (left) and after (right) the treatment of *D. dao* at 100µg/mL

Table 6. Hemoglobin concentrations of CAM

Treatment group	Concentration (µg/mL)	Hb concentration (%)
Distilled water (-)	30	1.4462
	50	2.4078 0.2902
	100	0.2098
Quercetin (+)	100	1.4836
D. dao	10	2.6455 2.2704 3.1716
	30	0.2179 0.5798
	50	1.7791
	100	1.6949
Z. talanai	10	1.1608 0.1685
	30	1.9177

Appendix D

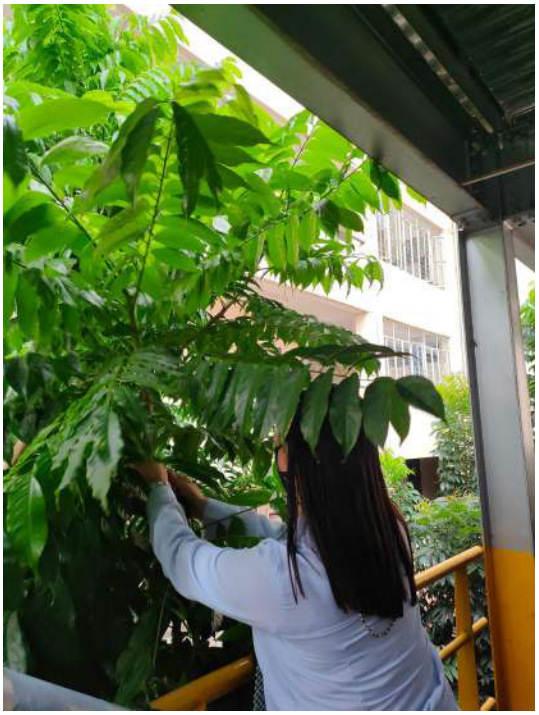


Figure 27. Dao collection in AUF

Figure 28. Talisay collection in Mansfield

Figure 29. Balakat collection in Our Lady of Grace Parish

Figure 30. Cutting of leaves

Figure 31. Cleaning of leaves

Figure 32. Drying of leaves

Figure 33. Crushing and grinding of leaves

Figure 34. Soaking of leaves

Figure 35. Filtration of leaves

Figure 36. Pressing of leaves for herbarium

Figure 37. Solvent system preparation

Figure 38. Viewing TLC plates under UV light.

Figure 39. Spray reagents for phytochemical screening

Figure 40. Rotation of eggs

Figure 41. Incubation of eggs

Figure 42. UV sterilization of laminar flow hood

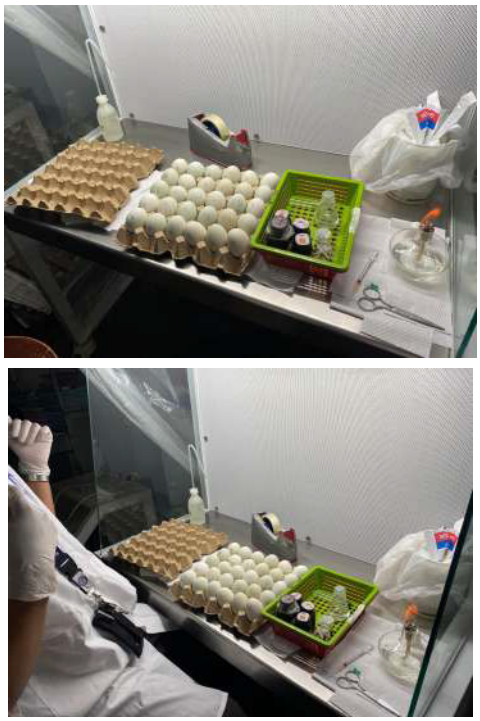


Figure 43. Setup in cleaning, marking, and administering treatments to eggs

Figure 44. Centrifuge setting for Drabkin reagent test

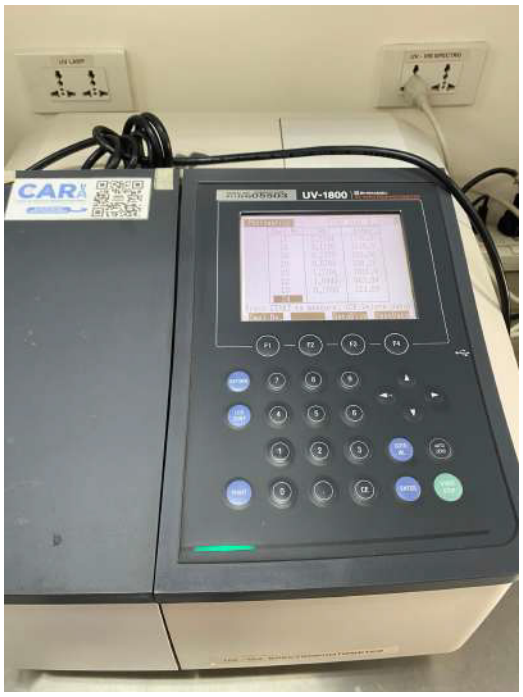


Figure 45. UV spectrophotometer setting for Drabkin reagent test