Valeology: International Journal of Medical Anthropology and Bioethics
(ISSN XXXX-XXXX) VOLUME 01 ISSUE 01, 2023

ANTI-ANGIOGENIC PROPERTY OF Ficus benjamina and Carmona retusa LEAF EXTRACTS USING Anas platyrhynchos CHORIOALLANTOIC MEMBRANE (CAM) ASSAY

Philip Andrew D. Sajise
Cyrene C. Serafica
Ralph Wency T. Tuma-ob
Mikko Jan D. Lopez, EdD
Regional Science High School for Region VI, Old Buswang, Kalibo, Aklan
Division of Aklan

Abstract:

Angiogenesis is the formation of new blood vessels that plays a key role in wound healing, reproduction, embryonic vascular development, and replacement of damaged body tissues; however, it is also present in pathophysiological conditions such as cancer and cardiovascular diseases, reasons like these demand for more researches that aim for cure and prevention. This where anti-angiogenesis comes into the picture as it inhibits the above-mentioned conditions. Medicinal plants like, Ficus benjamina and Carmona retusa that are commonly found in gardens were observed to have these anti-angiogenic properties. In this study, various concentrations of Ficus benjamina and Carmona retusa leaf extracts were applied on Anas platyrhynchos Chorioallantoic Membrane as it exhibited the essential features of angiogenesis. Each leaf extracts was conducted to a control group with three different concentrations (100mg/disc, 10mg/disc, and 1mg/disc); Retinoic acid being the positive control and distilled water was used as a negative control. To measure the anti-angiogenic effect of each treatment on the CAM, ImageJ, a software was used to count the number of blood vessel branches and its length. One-way ANOVA and Tukey-HSD were used to analyze the results. It showed that the treatment with the highest concentration of Ficus benjamina inhibits angiogenesis compared to other concentrations and treatments with Carmona retusa. There was a significant difference among the different treatments in terms of the number of blood vessels branches and branches' length. Such results stated that both leaf extracts inhibit angiogenesis. All inferential tests were set at 5% alpha level of significance.

Keywords: Angiogenesis, Chorioallantoic Membrane, Ficus benjamina and Carmona retusa, Leaf Extract

Introduction

Angiogenesis, the formation of new capillaries from pre-existing vessels, is a natural bodily process and is essential for several normal physiological events such as the body's replacement of damaged tissues, wound healing, embryonic vascular development, and reproduction (Katsi et al., 2014). Traditionally, *Ficus benjamina* tree is known for its medicinal properties and uses in treating various diseases. The leaves, fruits and its bark were reported to have various bioactive constituent such as triterpenes, alkaloids, ascorbic acid and flavonoids, lactose, quercetin, caffeic acid and stigmasterol (PharmaTutor Edu Labs, 2018-2019; Stuartxchange.com, 2019). According to Principe & Jose (2002), *Carmona retusa* is usually several-stemmed woody plant locally known as "Tsaang-Gubat" and one of the top ten approved medicinal plants in the Philippines. In the study conducted by Penecilla (2008), it was found out that "Tsaang-Gubat" has high potential in fighting growth and multiplication activities on cancer cells.

Due to lack of researches in verifying the anti-angiogenic properties of both plant samples, this research study was conducted to determine if the leaf extracts of *Ficus benjamina* and *Carmona retusa* that can inhibit angiogenesis. Through this, the current knowledge of the researchers and those who are concerned about angiogenesis and angiogenic drugs will be refined. In relation, the study was focused to test the effectiveness of *Ficus benjamina* and *Carmona retusa* leaf extracts that help in inhibiting angiogenesis.

This study was limited to the different treatments with different concentrations of *Ficus benjamina* and *Carmona retusa* leaf extracts to identify the number of blood vessel branches and the blood vessel branches' length. This study could help in decreasing the rate of these pathophysiological conditions by inhibiting angiogenesis and could be used as basis in formulating affordable, safe, and new medicines.

Literature Review

Angiogenesis or neovascularization is the process that creates new blood vessels, forming extensions for the currently present vasculature. The principal cells referred are identified as endothelial cells, which is connected to all blood vessels and is composed mostly of the entire capillaries. To form new blood vessels, endothelial cells break through the basement membrane to escape its original position. Once this process is successfully taken, endothelial cells move towards angiogenic stimulus which might come from tumor cells, activated lymphocytes, or wound-associated macrophages. While undergoing through different areas, endothelial cells rapidly increase the cell production that is essential in making a new vessel. Right after the cell production, the product needs to reorganize into a patent three-dimensionally tubular structure. Every element, basement membrane disruption, cell migration, cell proliferation, and tube formation can be a possible element for studying, and each can be tested through in vitro. However, studies that test angiogenesis need a more holistic assessment and several in vivo assays to develop a more realistic appraisal of the angiogenic results undergoing the process of in vitro (Auerbach et al., 2003)

The formation of new blood vessels which is fundamental in neoplasm is a process known as angiogenesis. Changes in these processes lead to different malignant, inflammatory, ischaemic, infectious and immune disorders. Recent studies have approved that anti-angiogenic agents are treatments for blindness and cancer. Angiogenesis research will create a big impact in the field of medicine in the years to come with an estimate of more than 500 million people to benefit from treatments using anti or pro-angiogenic agents (Carmeliet, 2005).

Angiogenesis is an important part in anatomical physiological processes and also in the formation of a new vasculature linked to the progression of cancer. Among the different animal model systems that are used to study the procedures behind angiogenesis, the embryo of the chick has been a commonly used test subject in determining the potential of angiogenesis in purified factors of intact cells. The chorioallantoic membrane (CAM), a specialized avian embryo tissue that is highly vascularized is used as an indicator of the anti-angiogenic or pro-angiogenic properties of test compounds. In this chapter, the different basic chick embryo CAM models of angiogenesis have been described. The study gave emphasis on the model system that employs a three-dimensional (3D) collagen grafts seeded on the CAM, referred herein as on plants. This collagen on plant model intends a clear logical quantity of angiogenesis and a higher understanding of the cellular and biochemical processes by which the identified cells of various ancestry or refined stimulative molecules catalyze or restrain angiogenesis (Deryugina and Quigley, 2009).

Chorioallantoic membrane (CAM) assay is often used in studying vascular formation, invasion of cell tumor and metastasis. There are many advantages in using the CAM model, such as (a) the CAM is greatly vascularized that highly advances its edge in tumor cell grafting; (b) it is easily reproduced and commonly available; (c) minimal expenses are expected, and finally (d) being identified as a closed system, small peptides as an experimental molecule tend to have longer half-life compared to animal models, making it a high potent material in studying anti-metastatic compound which is studied only in small numbers. The CAM is composed of different layers of epithelium; the ectoderm at the air interface, mesoderm (or stroma) and endoderm at the interface containing the allantoic sac. Furthermore, the CAM contains extracellular matrix proteins (ECM) such as fibronectin, laminin, collagen type I and integrin $\alpha v\beta 3$. The physiognomy of these extracellular matrix proteins replicates the physiological cancer cell conditions (Elder et al., 2012).

The genus Ficus is known to have 800 species worldwide and ten of these species are found in the Philippines. Six of these share a common name “Balete”, namely Ficus benjamina (salisi), Ficus elastica (Indian rubber tree), Ficus indica (baleteng-baging), Ficus payapa (payapa), Ficus retusa (marabutan), and Ficus stipulosa (botgo). Ficus 16 benjamina also known as weeping fig is a huge multipurpose tree that can grow 15-30 meters tall and its trunk can be 30-60 cm in diameter. Weeping fig is also the official tree of Bangkok, Thailand and are also found in India, China, Nepal, Myanmar, Australia, Vietnam, Malaysia, Pacific Islands, and the Philippines. It is also used as an avenue of tree shades in cities, and ropes made from its fibers in most provinces (Stuartxchange.com, 2019).

According to the studies conducted by Konyalioglu et.al, (2005) and Cuzzocrea et.al (2001), free radical scavenging activity of medicinal plants have shown that it is responsible for their anti-inflammatory effect. Ficus carica methanolic extract also contained high phenolic content that indicates high antioxidant properties that plays a big role in inhibiting angiogenesis. In the study conducted by Esteraf-Oskouei et.al (2015), it was concluded that Ficus carica methanolic extract has potent anti-inflammatory activities at the level of cell migration, exudate volume, tissue weight, and angiogenesis as well as in the content of proinflammatory mediators such as PGE2, TNFα, and VEGF levels which were more pronounced when it was injected locally as opposed to systemically.

A usually several-stemmed woody plant called “Tsaang Gubat” by the locals is native in the Philippines considering the condition in the country. According to Principe & Jose (2002), it has been proven to have medicinal properties which are less pricy and more natural than combined substances in drugs, it has been allowed by the Philippine Department of Health-Philippine Institute of Traditional Alternative Health Care (DOH-PITAHC). Moreover, it is found out that particular Carmona species is proven to be useful in several illnesses (CP, M, & Kumar, 2012). It is proven that this kind of plant has different properties that aid various illnesses (Chandrappa et al., 2013; CP, Govindappa, Nv, & Channabasava, 2012; CP, M, et al., 2012; Lam, 2012; Principe & Jose, 2002; Reglos-zara et al., 2010; Starr, Starr, & Loope, 2003; Villaseffor et al., 1992). This kind of species contains high potential phytochemical medicinal property that is used in treating different sicknesses (Chandrappa et al., 2013; CP, Govindappa, et al., 2012; CP, M, et al., 2012; Reglos-zara et al., 2010; Villaseffor et al., 1992).

Methodology

Research Design

The Scientific Experimental Design using Completely Randomized Design was used in this study

Materials

The materials used in this study were the ff: 1L beakers, Amber bottles, syringe, disposable containers, filter paper, weighing scale, blender, funnel, scissors, gloves, face masks, goggles, plastic wrap, labeling tape, markers, tissue, paper bags, garbage bags, Petri dish, spatula, paper discs, forceps, tuberculin syringe, egg trays, hair nets, micropore tape, plastic cups, laboratory gowns, mobile phones, towel, cotton balls, and droppers. The reagents used were as follows: 1L distilled water, 1.6L methanol from Patagonian Enterprises. *Ficus benjamina* and *Carmona retusa* leaves were obtained from Barangay Poblacion, Nabas, Aklan. *Anas platyrhynchos* eggs were obtained from New Buswang, Kalibo, Aklan.

Procedures

Collection of Specimens. Collection of fresh matured leaves of *Ficus Benjamina* and *Carmona retusa* were done at Brgy. Poblacion, Nabas, Aklan. Stalk from the obtained leaves were removed, washed, and air-dried for 48 hours at a room temperature.

Preparation of Crude Extracts. 100 grams of fine, dried powdered leaves of each plant samples were macerated separately in a mixture of 1000 mL consisting of 80% aqueous methanol for thirty-six (36) hours at a room temperature and stored in a closed cabinet. Extracts were then filtered using filter paper and were concentrated at 50°C using a rotary evaporator at the University of San Agustin–Gregor Mendel Research Laboratory, Iloilo City.

Collection and Incubation of Anas platyrhynchos Eggs. Five-day old *Anas platyrhynchos* eggs were obtained from New Buswang, Kalibo, Aklan. Eggs were then cleaned using a damp cloth to remove dirt. Egg Candling was performed to determine if the eggs were fertilized. Cracked, misshapen, soiled, or unusually small or large eggs were rejected. Accepted eggs were incubated for five (5) days at the Pharmaceutical Biological Science Laboratory of Saint Gabriel College.

Preparation of Treatments. Immediately after windowing the egg, the treatments were applied to the specimen through a 6mm paper disc that can only hold a maximum of 0.01mL solution. Extract (mg) was dissolved in 5mL of distilled water, then measured into 0.01mL using a tuberculin syringe and wet the paper disc. The following treatments were applied inside a UV lit fume hood:

Different Treatments of Ficus benjamina, Carmona retusa, Positive, and Negative Controls with Different Concentrations

Treatments	Concentrations		
Ficus benjamina	Treatment 1: 100 mg/disc	Treatment 2: 10 mg/disc	Treatment 3: 1 mg/disc
Carmona retusa	Treatment 1: 100 mg/disc	Treatment 2: 10 mg/disc	Treatment 3: 1 mg/disc
Positive Control (Retinoic acid)	Treatment 1: 100 mg/disc	Treatment 2: 10 mg/disc	Treatment 3: 1 mg/disc
Negative Control	Treatment 1: 100 mg/disc	Treatment 2: 10 mg/disc	Treatment 3: 1 mg/disc

Windowing. The ten-day incubated eggs were candled using a concentrated light source to locate the air sac and identify the prominent Y-shaped blood vessels. Using spatula and forceps, part of the shell was removed where the air sac is located. Using a dropper, excess fluids were removed, and the paper discs were administered on the Y-shaped blood vessel in the eggs. The eggs were then closed using a micropore and was placed back in the incubator for 3 days of re-incubation.

Transferring to Petri dish. Three days after application of treatments, the eggs were taken out, opened, and transferred to Petri dish. Then, a plastic ring of 1-inch in diameter was placed on the area around the Y-shaped part where the paper disc was planted on. This was done to limit the field of analysis.

Image processing and Software Analysis. A digital camera was used to capture the area of analysis on the CAM. A distance of 4 inches between the camera and the CAM during image taking was maintained for all assays. Auto focus was also enabled to ensure clarity of photos. Image processing was done to adjust all pictures to have uniform resolution and number of pixels. This was done at the quality control area of the Pharmaceutical Biological Science Laboratory of Saint Gabriel College. To eliminate to possibility of human error, the quantification of angiogenesis was made using the "Angiogenesis Analyzer" extension of the ImageJ software. The automatically processed data for number of branches and total length of branches were acquired using this software. ImageJ was downloaded online for free.

Findings and Discussion

Survival of the Incubated Eggs of *Anas platyrhynchos*

Table 1 below the survival of the incubated eggs of *Anas platyrhynchos* with the different applied treatments of different concentrations of *Ficus benjamina* (100mg/disc, 10mg/disc, 1mg/disc) and the different concentrations of *Carmona retusa* (100mg/disc, 10mg/disc, 1mg/disc).

Table 1. Survival of the Incubated Eggs of Anas platyrhynchos

Treatments	**Survival of the Incubated Eggs of Anas platyrhynchos**					
	Trial 1		**Trial 2**		**Trial 3**	
	R1	**R2**	**R3**	**R4**	**R5**	**R6**
100 mg/disc *Ficus benjamina*	Survived	Dead	Survived	Survived	Survived	Dead
10 mg/disc *Ficus benjamina*	Survived	Survived	Survived	Dead	Survived	Survived
1 mg/disc *Ficus benjamina*	Survived	Survived	Survived	Dead	Survived	Survived
100 mg/disc *Carmona retusa*	Survived	Survived	Survived	Survived	Survived	Survived
10 mg/disc *Carmona retusa*	Survived	Survived	Survived	Survived	Survived	Survived
1 mg/disc *Carmona retusa*	Survived	Survived	Survived	Survived	Survived	Survived
Positive Control	Survived	Survived	Survived	Survived	Survived	Survived

Negative Control	Survived	Survived	Survived	Survived	Survived	Survived

Results shows that four out of forty-eight *Anas platyrhynchos* eggs died after the three days of re-incubation. It was identified that the said four eggs were treated with different concentrations of *Ficus benjamina*. Two eggs died from the first treatment with a concentration of (100mg/ 5mL/ disc), one egg died from the second treatment with a concentration of (10mg/ 5mL/ disc), one egg died from the third treatment with a concentration of (1mg/ 5mL/ disc).

Table 1 states that there is a total of four *Anas platyrhynchos* eggs that were declared dead after three days of re-incubation upon the application of treatments, there were two coming from Treatment 1 with a 100mg/disc solution and one coming from both Treatment 3 with a 1mg/disc solution and Treatment 2 with a 10mg/disc solution deriving to come up with an idea that higher solutions of *Ficus benjamina* pose higher lethality risk on the CAM.

Number of Blood Vessel Branches in Different Treatments using Anas platyrhynchos CAM Assay

Table 2 shows the average number of branches of the incubated eggs of *Anas platyrhynchos* with different applied treatments of different concentrations of *Ficus benjamina* (100mg/disc, 10mg/disc, 1mg/disc) and the different concentrations of *Carmona retusa* (100mg/disc, 10mg/disc, 1mg/disc).

Table 2. Total and Mean Results of the Number of Blood Vessel Branches in Different Treatments using Anas platyrhynchos CAM Assay.

Treatments	**Number of Blood Vessel Branches using Anas platyrhynchos CAM Assay**							
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**Total**	**Mean**
100 mg/disc *Ficus benjamina*	84	X	94	87	101	X	366	61
10 mg/disc *Ficus benjamina*	142	132	139	X	137	141	691	115.17
1 mg/disc *Ficus benjamina*	173	164	157	X	174	153	821	136.83
100 mg/disc *Carmona retusa*	121	130	119	104	128	94	696	116
10 mg/disc *Carmona retusa*	151	147	144	148	149	147	886	147.67

1 mg/disc *Carmona retusa*	202	196	174	186	188	177	1123	187.17
Positive Control	16	82	49	4	70	7	228	38
Negative Control	205	208	213	234	235	257	1352	225.33
Grand Total	1094	1059	1089	763	1182	976	**6163**	
Grand Mean	136.75	132.375	136.125	95.375	147.75	122		**128.40**

Results show that the Treatment 1 (100mg/disc) of *Ficus benjamina* had the mean number of branches of 61, Treatment 2 (10mg/disc) of *Ficus benjamina* had the mean number of branches of 115. 17, Treatment 3 (1mg/disc) of *Ficus benjamina* had the mean number of branches of 136.83.

Treatment 1 (100mg/disc) of *Carmona retusa* had the mean number of branches of 116, Treatment 2 (10mg/disc) of *Carmona retusa* had the mean number of 147.67, Treatment 3 (1mg/disc) of *Carmona retusa* had the mean number of 187.17.

Positive Control had the mean number of 38, Negative Control had the mean number of 225.33, the highest out of all the treatments used. Results also showed the Grand Total number of branches of 6163 and have the Grand Mean of 128.40.

It also states that in terms of the number of branches in *Anas platyrhynchos* Chorioallantoic Membrane (CAM) assay after 72 hours, Treatment 1 of *Ficus benjamina* (100mg/disc) has the lowest mean, followed by Treatment 2 of *Ficus benjamina* (10mg/disc), Treatment 1 of *Carmona retusa* (100mg/disc), Treatment 3 of *Ficus benjamina* (1mg/disc), Treatment 2 of *Carmona retusa* (10mg/disc), and lastly Treatment 3 of *Carmona retusa* (1mg/disc). The leaves of *Ficus benjamina* contains caffeic (PharmaTutor Edu Labs, 2018-2019; Stuartxchange.com, 2019), while leaves of *Carmona retusa* contains polyphenolic compounds (Salas & Totaan, 2015), that makes *Ficus benjamina* able to decrease the number of blood vessels compared to *Carmona retusa*.

Number of Blood Vessel Branches in Different Treatments using *Anas platyrhynchos* CAM Assay

Table 3 in the succeeding page shows the significant difference results between the number of blood vessel branches in different treatments using *Anas platyrhynchos* CAM assay with the different applied treatments of different concentrations of *Ficus benjamina* (100mg/disc, 10mg/disc, 1mg/disc) and the different concentrations of *Carmona retusa* (100mg/disc, 10mg/disc, 1mg/disc) using one-way ANOVA.

Table 3. Significant Difference Results between the Number of Blood Vessel Branches in Different Treatments using Anas platyrhynchos CAM Assay.

Sources	SS	df	MS	F	Sig.
Treatment	158014.313	7	22573.473	15.186	*0.000
Error	59459.167	40	1486.479		
Total	217473.479	47			

*p<0.05, significant difference @5% alpha level of significance

Using One Way Analysis of Variance (ANOVA), there is a significant difference between the numbers of blood vessel branches in different treatments using *Anas platyrhynchos* CAM assay. (F=15.186, p=0.000).

Yhe effectivity in the number of branches of the blood vessels shown in Table 3 shows that there is a significant difference between the number of blood vessel branches in different treatments using *Anas platyrhynchos* CAM assay. It means that Treatment 1 of *Ficus benjamina* with the concentration of 100mg/disc is the most effective among all concentrations of the leaf extracts.

Branch Length (pixel) using *Anas platyrhynchos* CAM Assay

Table 5 shows the average of branches' length of the incubated eggs of *Anas platyrhynchos* with the different applied treatments with the different concentrations of *Ficus benjamina* (100mg/disc, 10mg/disc, 1mg/disc) and the different concentrations of *Carmona retusa* (100mg/disc, 10mg/disc, 1mg/disc).

Table 5. Branch Length (pixel) using Anas platyrhynchos CAM Assay

Treatments	**Branch Length (pixel) using Anas platyrhynchos CAM Assay**							
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**Total**	**Mean**
100 mg/disc *Ficus benjamina*	9493	x	10116	10170	10814	x	40593	6766
10 mg/disc *Ficus benjamina*	16664	13407	8763	x	12308	13429	64571	10761.83
1 mg/disc *Ficus benjamina*	15576	12113	12391	x	15933	10290	66303	11050.50
100 mg/disc *Carmona retusa*	11233	12230	9879	7521	14328	10908	66099	11017

10 mg/disc *Carmona retusa*	13081	11107	15733	11423	12390	12976	76710	12785.00
1 mg/disc *Carmona retusa*	13210	19376	14095	15261	12127	14773	88842	14807.00
Positive Control	2443	8366	7941	778	8150	1918	29596	4933
Negative Control	14072	16817	17343	14542	19368	19357	101499	16916.50
Grand Total	95772	93416	96261	59695	105418	83651	**534213**	
Grand Mean	11971.5	11677	12032.625	7461.875	13177.25	10456.375		**11129.44**

Data in Table 5 states that in terms of number of total branches' length in *Anas platyrhynchos* Chorioallantoic Membrane (CAM) assay after 72 hours, the Treatment 1 of *Ficus benjamina* (100mg/disc) exhibited the lowest mean, Treatment 2 of *Ficus benjamina* (10mg/disc), Treatment 1 of *Carmona retusa* (100mg/disc), Treatment 3 of *Ficus benjamina* (1mg/disc), Treatment 2 of *Carmona retusa* (10mg/disc), and lastly, Treatment 3 of *Carmona retusa* (1mg/disc). The leaves of *Ficus benjamina* contains caffeic (PharmaTutor Edu Labs, 2018-2019; Stuartxchange.com, 2019), while leaves of *Carmona retusa* contains polyphenolic compounds (Salas & Totaan, 2015), that makes *Ficus benjamina* able to decrease the blood vessel branches' length when compared to *Carmona retusa.*

Length of Branches in Different Treatments using *Anas platyrhynchos* CAM Assay

Table 6 shows the significant difference results between the numbers of blood vessel branches in different treatments using *Anas platyrhynchos* CAM assay with the different applied treatments of different concentrations of *Ficus benjamina* (100mg/disc, 10mg/disc, 1mg/disc) and the different concentrations of *Carmona retusa* (100mg/disc, 10mg/disc, 1mg/disc) using one-way ANOVA

Table 6. Significant Difference Results between the Length of Branches in Different Treatments using Anas platyrhynchos CAM Assay

Sources	SS	df	MS	F	Sig.
Treatment	644120902	7	92017271.7	5.752	*0.000
Error	639874194	40	15996854.9		

Total	1283995096	47

*p<0.05, significant difference @5% alpha level of significance

In terms of the effectivity in the branches length of the blood vessels shown in Table 6, using One Way Analysis of Variance (ANOVA). There is a significant difference between the numbers of pixels of branches' length in different treatments using *Anas platyrhynchos* CAM assay. ($F=5.752$, $p=0.000$). It means that Treatment 1 of *Ficus benjamina* with the concentration of 100mg/disc is the most effective among all concentrations of the leaf extracts.

Based on the results, *Ficus benjamina* plant has more anti-angiogenic capability compared to *Carmona retusa* in terms of the growth of number of blood vessel branches and number of pixels consumed by the branches' length.

CONCLUSIONS

Based on the data, the researchers conclude that higher concentrations of *Ficus benjamina* pose higher lethality risk on the *Anas platyrhynchos* egg Chorioallantoic Membrane (CAM) assay while on the other hand, the different concentrations of *Carmona retusa* leaf extracts gave no bearing on the survival rate of the *Anas platyrhynchos eggs*.

Therefore it can conclude based on data that in terms of the numbers of branches and branches' length in *Anas platyrhynchos* Chorioallantoic Membrane (CAM) assay after 72 hours, the growth of blood vessels with all of *Ficus benjamina* and *Carmona retusa* extract concentrations are lower compared to the Negative Control; therefore, *Ficus benjamina* and *Carmona retusa* leaf extracts diluted in distilled water inhibits the growth of blood vessels more effectively than distilled water alone.

Treatment 1 of *Ficus benjamina* with the concentration of 100mg/disc is more effective compared to other concentrations of *Ficus benjamina* and concentrations of *Carmona retusa* in terms of blood vessel growth based on the data gathered using One Way Analysis of Variance (ANOVA) on Tables 3 and 6, The tables suggests that *Ficus benjamina* has more capabilities in inhibiting angiogenesis.

Results show that there is a significant difference between the results of the treatments. The tables strengthen the data that both *Ficus benjamina* and *Carmona retusa* leaf extracts have anti-angiogenic properties

References

1. Aisha A., et al. (2009). Cytotoxic and Anti-angiogenic Properties of the Stem Bark Extract of Sandoricum koetjape. Int J Can Res. 5 (3): 105-114.
2. Auerbach, R., et al. (2003). Angiogenesis Assays: A Critical Overview. Retrieved June 23, 2019 from http://clinchem.aaccjnls.org/content/49/1/32
3. Buyun L. (2016). The antimicrobial potential of ethanolic extract from Ficus benjamina

4. L. leaves. Retrieved June 23, 2019 from https://www.researchgate.net/publication/301681780_The_antimicrobial_potential_of_ethanolic_extract_from_Ficus_benjamina_L_leaves
5. Carmeliet, P., Jain, R. (2000). Angiogenesis in cancer and other diseases. Retrieved June 22, 2019 from https://www.nature.com/articles/35025220
6. Carmiliet, P. (2005). Angiogenesis in life, disease and medicine. Retrieved June 23, 2019 from https://www.nature.com/articles/nature04478
7. Compton, S. (2003). What is Angiogenesis? - Definition & Factors. Retrieved June 23, 2019 from https://study.com/academy/lesson/what-is-angiogenesis-definition- factors-quiz.html
8. Elder. F., et al. (2012). Chick Chorioallantoic Membrane (CAM) Assay as an In Vivo Model to Study the Effect of Newly Identified Molecules on Ovarian Cancer Invasion and Metastasis. Retrieved June 23, 2019 from https://www.mdpi.com/1422-0067/13/8/9959/htm
9. Eteraf-Oskouei T., Allahyari S., Akbarzadeh-Atashkhosrow A., Delazar A., Pashaii M., Gan S.H., Najafi M. 2015. Methanolic Extract of *Ficus carica Linn*. Leaves Exerts Anti-angiogenesis Effects Based on the Rat Air Pouch Model of Inflammation. Evidence-Based Complementary and Alternative Medicine. 2015:1-9.
10. Gilman E., Watson D. (1993). Ficus benjamina Weeping Fig[1] . Retrieved June 21, 2019 from http://hort.ufl.edu/database/documents/pdf/tree_fact_sheets/ficbena.pdf
11. Gullino, P. (1978). JNCI: Journal of the National Cancer Institute, Volume 61, Issue 3, September 1978, Pages 639–643. Retrieved June 22, 2019 from https://academic.oup.com/jnci/ article/61/3/639/957547
12. Hasti S., Mora E., Utami R., Yulis L.U. 2014. Subchronic Toxicity of Ficus benjamina L. Leaves Ethanol Extract on the Liver Function of White Mice. Procedia Chemistry. 13(2014): 204-208.
13. Katsi V., Psarros C., Krokodis M., Vamvakou G., Tousoulis D., Stefanadis C., Kallikazaros
14. Anti-Angiogenic Therapy and Cardiovascular Diseases: Current Strategies and Future Perspectives. Anti-Angiogenesis Drug Discovery and Development. 2014:268-308.
15. Mignatti P., and Rifkin., D.B. “Plasminogen activators and matrix metalloproteinases in angiogenesis,” Enzyme and Protein, vol. 49, no. 1–3, pp. 117–137, 1996.
16. Morgan and Claypool Life Sciences (2010). Angiogenesis. Retrieved June 23, 2019 from https://www.ncbi.nlm.nih.gov/books/NBK53238/
17. National Cancer Institute (2018). Angiogenesis Inhibitors. Retrieved June 23, 2019 from https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/angiogenesis-inhibitors-fact-sheet
18. Pawlus A., Cartwright C., Vijeswarapu M., Liu Z., Woltering E., Newman R. 2008. Anti- angiogenic activity from the latex of Ficus carica (Fig). J Ethnopharmacol.74(09).
19. Pepper M.S., “Role of the matrix metalloproteinase and plasminogen activator-plasmin systems in angiogenesis,” Arteriosclerosis, Thrombosis, and Vascular Biology, vol. 21, no. 7, pp. 1104–1117, 2001.
20. PharmaTutor Edu Labs . (2018-2019). A review on biological and phytochemical properties of ficus species. Retrieved June 21, 2019 from https://www.pharmatutor.org/articles/review-biological-phytochemical-properties-ficus-species?page=2%2C3
21. Planta Med. (2008). Anti-angiogenic activity from the fruit latex of Ficus carica (Fig).

22. Retrieved June 23, 2019 from https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0028-1084095