Anti-angiogenic effect of flavonoid content of *Allium cepa L. var. ascalonicum* Backer (onion) skin on chick chorioallantoic membrane

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Abstract: Angiogenesis is a natural process of forming new blood vessels in the body; however, excessive proliferation of blood vessels favors tumor growth. Since tumor growth and metastasis require angiogenesis to take place, anti-angiogenic therapy is being targeted as an approach to cancer treatment. Flavonoid content from *Allium cepa L. var. ascalonicum* Backer (onion) skin was evaluated for its angiogenic inhibitory effect using shell-less chick chorioallantoic membrane (CAM) assay. Twenty-five one-day old fertile hen eggs were classified into groups that received 80% methanol (negative control), and varying concentrations (25%, 50%, 75%, and 100%) of flavonoids (treatment groups) on the sixth day of incubation. The angiosuppressive effect of the extract was evaluated by counting the average number of branch points of the CAM on the eighth day of incubation. Results showed well-developed and branched blood vessels in the negative control while in the treatment group, decreasing number of blood vessels were observed as the concentration of flavonoid content increases as also revealed in the visual assessment and photography results. Using Tukey HSD and Dunnett test, the angiogenic effect of flavonoid content of *A. cepa* on the proliferation of blood vessels to chicken embryos was proven to be statistically significant. Significant differences were observed between the negative control group and the treatments used. It showed that the greater concentration applied the lesser the branched points observed. This proves that the flavonoid content of *A. cepa* skin has an anti-angiogenic effect; thus, it could be a potential agent against cancer, through the inhibition of blood vessel proliferation.

Keywords: *Allium cepa*, angiogenesis, chorioallantoic membrane

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INTRODUCTION

Angiogenesis is a complex process that involves the activation, proliferation, and directed migration of endothelial cells to form new capillaries from existing blood vessels. This sprouting of capillaries from preexisting vessels occurs during embryonic development but is almost absent in adult tissues except in wound healing. Pathologic angiogenesis is characterized by the persistent proliferation of endothelial cells and is a prominent feature of a number of diseases, including rheumatoid arthritis, psoriasis, and proliferative retinopathy (Oklu, Walker, Wicky, and Hesketh, 2010).

According to Bisht, Dhasmana, and Bisht (2010), both angiogenesis insufficiency as well as excess can lead to various disorders. Insufficient angiogenesis is a characteristic of many disorders including ischemic tissue injury or cardiac failure. Most of these conditions are associated with decreased levels of vascular endothelial growth factor. On the other hand, excessive vascular growth contributes to numerous disorders. The already known disorders associated with it are cancer, arthritis, psoriasis, and blinding retinopathy. Even infectious diseases are also angiogenic. Viral and bacterial pathogens carry angiogenic genes or induce the expression of angiogenic genes in the host.

Cancer is a class of disease characterized by out of control cell growth which harms the body when damaged cells proliferate uncontrollably to form lumps or masses of tissue called tumors (Crosta, 2008). It is a condition that affects people all over the world (Silverstein, Silverstein, and Nunn, 2006). There were an estimated 14.1 million cases around the world in 2012. Of these, 7.4 million cases were in men and 6.7 million in women according to World Cancer Research Fund International (2012). In the Philippines, approximately 98,200 people are newly diagnosed of cancer every year and about 59,000 die from it (CancerIndex, 2012). In year 2013, Philippines had the highest incidence of breast cancer in the continent and an estimated 3 out of 100 Filipino women will contract the disease before age 75 as stated in the community bulletin board of GMA News Online (2013).

Angiogenesis has been an appealing target for anticancer drugs for 30 years, but it is only recently that this promise has borne some fruits. Anti-angiogenic therapy targets vascular growth within tumors, with the aim of suppressing tumor growth and metastasis. Current anti-cancer chemotherapeutic agents used in the clinical setting indiscriminately target all rapidly dividing cells and therefore can cause severe adverse effects such as immunosuppression, intestinal problems and hair loss. In comparison, anti-angiogenic reagents theoretically have fewer side effects (Kubota, 2011). There are now over 30

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angiogenesis inhibitors currently in clinical trials for the treatment of malignancy. These drugs appear to have a cytostatic rather than cytotoxic effect, leading to tumor dormancy. The available data suggest that anti-angiogenic drugs work best in conjunction with chemotherapy. Their development also involves the identification and management of a new range of toxicities (Clarke and Sharma, 2006). After more than three decades of intensive research, there is now proof that antiangiogenic therapy, especially when combined with chemotherapy, results in increased survival in patients suffering from advanced solid tumors. Clinical benefit of antiangiogenic therapy could be possibly enhanced if earlier stages of malignancy would be treated or antiangiogenic strategies would be applied in an adjuvant setting (Eichhorn, Kleespies, Angele, Jauch, and Bruns, 2007).

To analyze the mechanisms underlying normal and pathological angiogenesis, numerous in vivo angiogenic assays have been established employing different species of laboratory animals, including mammals (mouse, rat, hamster, and rabbit), birds (chicken and quail), and fish (mainly zebra fish). The use of chick embryo models for angiogenic studies is facilitated by the existence in avian species of a specialized respiratory tissue, named the chorioallantoic membrane (CAM) that allows for gas exchange between the embryo and the atmosphere surrounding the egg and in effect performs the function of a lung during embryonic life (Deryugina and Quigley, 2009).

Chick embryo chorioallantoic membrane (CAM) is an extraembryonic membrane serving as a gas exchange surface. It is formed on day 4 of incubation by fusion of the chorion and allantois (Ribatti, 2010). The CAM contains extracellular matrix proteins such as fibronectin, laminin, collagen type I and integrin $\alpha v\beta_3$ which mimic the physiological cancer cell environment (Lokman, Elder, Ricciardelli, and Oehler, 2012). The chorioallantoic membrane assay is an emerging model of tumor progression using the embryonic chicken (Scanlon, Inglehart, and D'Silva, 2013). The CAM may also be used to verify the ability to inhibit the growth of capillaries by implanting tumors onto the CAM and by comparing tumor growth and vascularization with or without the administration of an anti-angiogenic molecule (Ribatti, 2010).

Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain. Drug discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been

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applied towards combating cancer (Balunas and Kinghorn, 2005). Several polyphenols, especially those derived from tea, grapes or red wine, inhibit angiogenesis when administered orally. The discovery of these compounds as angiogenesis inhibitors has shed light on the mechanisms behind the health benefits of natural products, which are rich in these components (Losso, Bagchi, and Shahidi, 2007).

Allium cepa L. (onion) belongs to the lily family Alliaceae (Kabura, Musa, and Odo, 2008). Onion plant grows about 2 feet tall and bears an underground globular stem, which consists of modified leaves arranged in whorls. There are many cultivar onions grown around the world. The average crop takes about three to four months time. Sharp, pungent smell of onions is due to its sulfur compound, *allyl propyl disulphide*. Spanish red onions are generally less strongly flavored than white or brown varieties, which makes them ideal for use in raw salads (Rudrappa, 2015). Onion is highly valued for its therapeutic properties and is known to help guard against many chronic diseases (Kumar, Bhowmik, Biswajit, Chiranjib, and Tiwari, 2010). Studies have shown that the extracts of onion leaves can slow down rate of colon cancer growth due to its flavonoid content (Evers, 2014). Onions are rich in different types of phenolics, mainly flavonols which were found to be predominant. Onions are found to be one of the world's oldest cultivated vegetables and it ranked second to tomatoes for being the most produced vegetable crop (Perez-Gregorio, Garcia-Falcon, Simal-Gandara, Rodriguez, and Almeida, 2009).

Figure 1. ***Allium cepa L. var ascalonicum***

In addition, onion bulbs are among the richest sources of dietary flavonoids and contribute to a large extent to the overall intake of flavonoids (Slimestad, Fossen, and Vagen, 2008). Flavonoids in food are generally responsible for color, taste, prevention of fat oxidation, and protection of vitamins and enzymes. They have extensive biological properties that promote human health and help reduce the

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risk of diseases. Flavonoids are also known to influence the quality and stability of foods by acting as flavorants, colorants, and antioxidants Fruits and vegetables having flavonoids have been reported as cancer chemopreventive agents. Consumption of onions and/or apples, two major sources of the flavonol quercetin, is inversely associated with the incidence of cancer of the prostate, lung, stomach, and breast. In addition, moderate wine drinkers also seem to have a lower risk to develop cancer of the lung, endometrium, esophagus, stomach, and colon. The critical relationship of fruit and vegetable intake and cancer prevention has been thoroughly documented (Kumar & Pandey, 2013).

Accordingly, various studies have shown the inverse relationship between consumption of onions and risk of developing cancer. In a study by Galeone, Giacosa, Franceschi, La Vecchia, and Levi (2006), moderate frequency of onion consumption demonstrated protective role against colorectal, laryngeal and ovarian cancers, while high frequency showed a more evident inverse relation against oral cavity and esophageal cancers. Also, a decreased risk of distal stomach cancer was observed with increasing onion consumption (Setiawan, Yu, Lu, Lu & Yu, 2005).

Another key point is that onion extract was shown to be unusually nontoxic. A dose as high as forty times that of the dose required to kill the tumor cells had no adverse effect on the host (Murray and Pizzorno, 2005). However, people with heartburn may note aggravation of symptoms of acid reflux (Pizzorno and Murray, 2012).

Currently, control of angiogenesis is recognized to have a potential therapeutic value against many diseases such as cancer (Adair & Montani, 2010). Phytochemicals are potential novel leads for developing anti-angiogenic drug. Thus, this study aims to prove that the flavonoid content of *A. cepa* can inhibit angiogenesis and may be used for preventing cancer. Furthermore, this will aid in promoting natural way of preventing cancer by utilizing the abundance of onion.

MATERIALS AND METHODS

Collection of Plant Material

The onions were purchased from the local market of Batangas City, Batangas. All the onions' skin were removed, cleansed with water and air-dried. The sample was submitted to the Botany Division of the National Museum for authentication.

Reagents

Standard quercetin and methanol were purchased from

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Belman Laboratories, Inc. Petroleum ether, diethyl ether, ethyl acetate, 7% sulphuric acid, aluminium chloride and sodium nitrite were purchased from DKL Laboratory Supplies.

Collection and Incubation of Eggs

Fifty-six fertilized chicken eggs were purchased from an egg supplier in Libjo, Batangas. Eggs were incubated for 72 hours prior to transferring to petri dish and administration of the different concentrations of flavonoids (Dohle, Pasa, Gustmann, Laub, Wissler, Jennissen & Dunker, 2009).

Extraction of Flavonoids

Ten kilograms of onions were purchased and the 200 mg dried outer skins were separated from the bulb, rinsed with water and air-dried at room temperature. After drying, the samples were pulverized using a blender. Only 50 mg of powdered sample was used and were then extracted with 80% methanol in a Soxhlet apparatus, afterwards it was filtered. The filtrate obtained from sample was extracted in petroleum ether, diethyl ether and ethyl acetate following the method of Subramanian and Nagarajan (1969). Petroleum ether fraction was discarded due to its being rich in fatty substances. Ethyl acetate fraction of sample was hydrolyzed further with 7% H_2SO_4 for 24 hrs and was then re-extracted with ethyl acetate. It was rotary evaporated and diluted with distilled water to obtain required concentrations (Yadav and Kumar, 2012). A dark red compound was obtained with respect to the starting crude material (Garib Naseri, Yahyavi & Arabian, 2007).

Determination of the Total Flavonoid Content

The determination of the flavonoid content of the sample was done by the Research specialists of the Chemistry and Biotechnology Section of the Department of Science and Technology, Laguna.

Aluminum chloride method was used for flavonoid determination. In this method, quercetin was used as standard and flavonoid contents were measured as quercetin equivalent. One milliliter of standard or extract solution (20, 40, 60, 80, 100 mg/l) was taken into 10ml volumetric flask, containing 4ml of distilled water to which 0.3ml of 5% $NaNO_2$ was added. After 5min, 0.3ml 10% $AlCl_3$ was added to the mixture. At the 6th minute, 2ml of 1M NaOH was mixed and the volume of the solution was made up to 10ml with distilled water. The absorbance was noted at 510nm using UV-Visible spectrophotometer (Sahu & Saxena, 2013).

Shell-less Chorioallantoic Membrane Assay

Prior to incubation, fertilized eggs were wiped with clean

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tissue and distilled water to remove any dirt, feather and excrement. The eggs were then incubated for 72 hours in an automatic incubator with a moving tray. Sixty to sixty-two percent humidity was kept with an incubation temperature of 37˚C. After the incubation, the eggs were viewed through egg candler to see where the blood vessel network was. Using an 80mm triangular magnetic stir bar, the crack was made on the opposite side of the blood vessel network to prevent disruption of the membrane. Each egg was carefully transferred to sterile petri dish. These ex ovo cultures were re-incubated at 37.5˚C in a static egg incubator. After 2 days, extract was applied onto the CAM then re-incubated once again (Dohle, Pasa, Gustmann, Laub & Wissler, 2009).

Sampling Treatment

The test specimens were divided into five groups, each consisting of five eggs (Deryugina and Quigley, 2009).

Group I : The eggs in this group served as control. They were administered with 20uL of eighty percent methanol.

Group II : The eggs in this group were administered with 25% flavonoid content of *A. cepa* skin.

Group III : The eggs in this group were administered with 50% flavonoid content of *A. cepa* skin.

Group IV : The eggs in this group were administered with 75% flavonoid content of *A. cepa* skin.

Group V : The eggs in this group were administered with 100% flavonoid content of *A. cepa* skin.

Visual Assessment and Photography

Examination of the CAM for angiogenesis was done 35 hours after extract was applied (Cao, 2006). Analysis and quantification of the blood vessels were made through the captured images. The images were prepared so that the saturation, light and contrast allowed a better resolution of the blood vessels. This is done with the use of ImageJ software (Melo-Reis, Andrade, Silva, Araujo, Pereira, Mrue, and Chen-Chen, 2010). The number of first-order blood vessels (i.e. smallest blood vessels) was counted manually in a clockwise direction on a quarter of the digitalized picture with at least 20 blood vessels. All observations were made blinded of the negative control group and treatment groups (Larger, Marre, Corvol & Gasc, 2004).

Statistical Analysis

This research made use of descriptive statistics such as frequency and mean to establish the number of blood vessel branch points. Dunnett's test (2-sided) was used to correlate the effect of each

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treatment when compared to negative control. Post Hoc Analysis using Tukey HSD method was used to determine the difference in the inhibition on blood vessel formation in varying concentrations.

RESULTS AND DISCUSSION

I. Plant Extract

Fifty (50) mg of powdered onion skins were utilized and extracted with methanol using Soxhlet apparatus. Approximately 100 ml of extract was obtained. The resulting extract was cloudy and dark red to brown in color as shown in figure 2. The same appearance was observed by Ambrose (2012) who used onion peel as a dyeing agent in her study.

Figure 2. Onion extraction using Soxhlet method

II. Flavonoid Extract

A series of fractionation using petroleum ether, diethyl ether and ethyl acetate was performed after Soxhlet extraction. The resulting extract from ethyl acetate which was further hydrolyzed was clear and mahogany red in color. Ethyl acetate fraction has the highest concentration of flavonoid among the other fractions according to Seyfi, Mostafaie, Mansouri, Arshadi, Mohammadi-Motlagh and Kiani (2010).

III. Total Flavonoid Content

The total concentration of flavonoids in the ethyl acetate fraction submitted was 2.03mg/ml. Onion, according to Lachman, Pronek, Heijtmankova, Dudjak, Pivec and Faitova (2003), contains flavonol quercetin 5-10 times higher in comparison with other vegetables.

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Figure 3. Ethyl acetate fraction of *A. cepa* extract

IV. Shell-less CAM Assay

Proper handling of the eggs was observed during the course of the study as evidenced by the successful transfer of eggs to the petri plates on the third day of incubation. The eggs showed healthy condition as demonstrated by undisrupted egg yolk, beating of the heart of the embryos and formation of the vascular network on the fifth day which was the day for experimental manipulation. Anti-angiogenic activity of the flavonoid content of *A. cepa* on chorioallantoic membrane assay was determined after 7 days of incubation. It was administered with 25, 50, 75 and 100% flavonoid concentrations.

Table 2 shows the average number of the blood vessels counted at different concentrations of flavonoid extracted from *A. cepa* skin.

Table 2
Number of Blood Vessels Observed at Different Flavonoid Concentrations Extracted from *A. cepa*

Concentration of Flavonoid	Average Number of Blood Vessels
Negative control	49.2
25%	37.0
50%	36.8
75%	32.4
100%	30.4

The number of blood vessels was counted manually on a quarter of the digitalized picture by counting branching points (Larger, et.al., 2004). The decrease in the number of blood vessels are seen in groups administered with flavonoid from *A. cepa* skin. An average of 49.2 blood vessels was counted on the negative control group. In Group 1 which was administered with 25% flavonoid concentration, an

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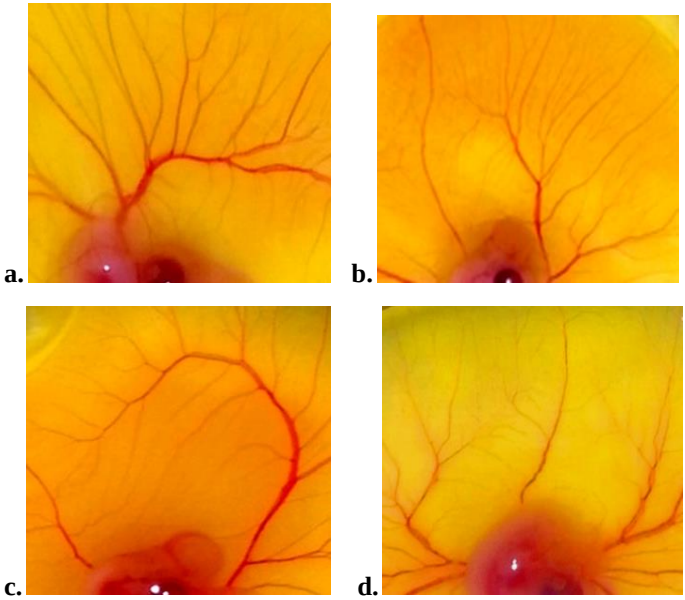
average of 37 blood vessels was formed. As the concentration of the flavonoids increased to 50%, 75% and 100%, decreasing average number of 36.8, 32.4 and 30.4 blood vessels were counted, respectively.

Based from the results, there were a decreasing average number of blood vessels formed as the concentration of flavonoids increase. This implies that increasing the concentration of the flavonoids could inhibit the proliferation of blood vessels. According to Thamilarashi, Mangalagowri and Winkins (2014) natural flavonoid myricetin inhibited blood vessel formation in a dose-dependent manner in-vivo. Histological crosssections of Myricetin treated CAM revealed reduced large and small newly synthesized blood vessels.

Macroscopic photographs of the chorioallantoic membrane are shown in Figure 4. Well-developed and branched blood vessels were prominent in the chick embryos of the negative control group. On the other hand, experimental groups treated with different concentrations of flavonoids from *A. cepa* developed fewer blood vessels which decrease as the concentration of flavonoids increase.



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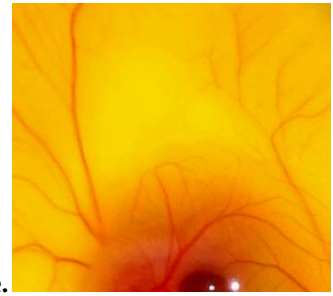
e. 

Figure 4. Representative pictures of the chorioallantoic membrane of the egg exposed to: a) 80%methanol b) 25% c) 50% d) 75% e) 100% flavonoid extract from onion

Anti-angiogenic property was observed with the experimental group with the embryos treated with 100% flavonoid concentration showing the major inhibition in the blood vessels. In this study, observation of anti-angiogenic activity of the flavonoids extracted from *A. cepa* is evident. This observation suggests that it may be a potential source of a therapeutic agent against cancer.

The comparison of the effects of the angiosuppressive activity of flavonoid content of *A. cepa* per group is shown in Table 3. It delineates the main difference between the results of each group in a study.

Table 3
Comparisons on the Effect of Each Treatment When Compared to Negative Control (Dunnett's test (2-sided))

(I) group	p-value	Interpretation
25%	.022	Significant
50%	.020	Significant
75%	.002	Highly Significant
100%	.001	Highly Significant

Significant at p-value <0.05

It was found out that there was a significant difference existing between the negative control and treated groups based on their effects on the inhibition of the formation of blood vessels when grouped according to treatment concentration since all the obtained p-values are less than 0.05.

Negative control against 25% flavonoid concentration gave a p-value of 0.022, while the concentrations 50%, 75%, and 100% gave p values of 0.20, 0.02 and 0.01, respectively. Every increase in flavonoid concentration showed a significant decrease in the number of blood

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vessels counted. This presents that the increasing concentration of the flavonoid significantly increases its action of inhibiting angiogenesis. Similarly, past studies have shown that flavonoids extracted from plants revealed increasing angiosuppressive activity as the concentration increases (Dai, Lu, Gao, Kang & Ma, 2013; Lirdprapamongkol, Kramb, Chokchaichamnankit, Srisomsap & Surarit, 2008).

The multiple comparison of each group with all the other groups based on the effect in the proliferation of blood vessels is presented in Table 4.

Table 4
Multiple Comparisons on the Effect of Each Treatment (Tukey HSD)

(I) group	(J) group	p-value	Interpretation
Negative control	25%	.045	Significant
	50%	.041	Significant
	75%	.004	Highly Significant
	100%	.001	Highly Significant
25%	Negative control	.045	Significant
	50%	1.000	Not Significant
	75%	.781	Not Significant
	100%	.488	Not Significant
50%	Negative control	.041	Significant
	25%	1.000	Not Significant
	75%	.807	Not Significant
	100%	.518	Not Significant
75%	Negative control	.004	Highly Significant
	25%	.781	Not Significant
	50%	.807	Not Significant
	100%	.987	Not Significant
100%	Negative control	.001	Highly Significant
	25%	.488	Not Significant
	50%	.518	Not Significant
	75%	.987	Not Significant

Significant at p-value <0.05

It came out that the different concentrations did not show significant difference when compared with each other. However, comparing negative control with other groups, it showed significant difference with 25% having a p-value of 0.045, 50% with p-value of

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0.041 and a highly significant difference with 75% and 100% flavonoid concentrations having p-value of 0.004 and 0.001, respectively.

These results imply that the minimum concentration of 25% can already inhibit angiogenesis. Increasing the concentration may increase the ability to inhibit angiogenesis; however, effect is not far different with the minimum concentration. This shows that the varying concentrations of flavonoid inhibit blood proliferation, but the minimum concentration may already be utilized.

Comparison on the effects of extract between each test group to the negative control group gave some different results to that of the comparison of one group against all the other groups. The treatment groups when compared with the negative control showed inhibition in a dose-dependent manner. However, treatment groups compared with each other showed no significant difference. Comparing these results with different bases creates a problem that cannot be escaped. Stating the multiple comparisons is not that essential but rather simply reporting all the data.

CONCLUSION

The research study was performed to observe the anti-angiogenic effect of *Allium cepa L. var. ascalonicum* Backer (onion) flavonoid content on shell less chorioallantoic membrane assay in different concentrations. This study proved that increasing concentration of the flavonoid has an anti-angiogenic capability. The statistical analysis presented a significant difference between the control and treated groups based on the effect on angiogenesis when grouped according to different concentrations. Based on the descriptive statistics presented, blood vessel proliferation decreases as the concentration increases, and 100% concentration revealed the greatest inhibitory capability in the development of blood vessels. However, an optimum concentration of 25% flavonoid from *A. cepa* is enough to inhibit angiogenesis. Nonetheless, this study concludes that flavonoid content of *A. cepa* skin is a possible source of chemotherapeutic agent against cancer through angiogenesis suppression.

RECOMMENDATION

The researchers recommend the determination of the anti-angiogenic activity of other plants containing flavonoids and compare the results with the current findings. Future researchers are also advised to submit the embryos for a histopathologic examination to determine if the extract can cause unwanted effects. Furthermore, the use of filter paper discs or rings in inducing the extract is recommended for better view of the effect of the extract on the blood vessels. Lastly, viewing and counting of blood vessels could be done microscopically and with

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the use of computer software designed for counting blood vessels for more accurate results. Different parameters may also be noted such as blood vessel density, CAM area or vascular percentage as to add significant findings on the effect of the extract used.

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REFERENCES

Adair, T. & Montani, J.P. (2010). *Angiogenesis.* CA: Morgan & Claypool Life Sciences. DOI:10.4199/C00017ED1V01Y201009ISP009

Ambrose, M.G. (2012, September 7). Natural Dyes – Red Onion Skins. *Folk Fibers* [Web log message]. Retrieved November 4, 2015 from http://www.folkfibers.com/blogs/news/6579481-natural-dyes-red-onion-skins

Balunas, M. & Kinghorn, A.D. (2005). Drug discovery from medicinal plants. *Life Sciences*, 78(5), 431- 441. Retrieved from http://dx.doi.org/10.1016/j.Its.2005.09.012

Bisht, M., Dhasmana, D.C., & Bisht, S.S. (2010). Angiogenesis: Future of pharmacological modulation. *Indian Journal of Pharmacology*, 42(1), 2-8. DOI:10.4103/02535-7613.62395

CancerIndex (n.d). Retrieved March 27, 2015 from www.cancerindex.org/Philippines

Cao, Y. (2006). Chapter 46: Angiogenesis assays. *Cell Biology*, p.373. USA : Elsevier Science.

Clarke, S. & Sharma, R. (2006). Angiogenesis inhibitors in cancer-mechanisms of action. *Experimental and Clinical Pharmacology*, 29(1), 9-12.

Crosta, P. (2008, September). What is cancer, what cause cancer. *Medical News Today*. Retrieved April 12, 2014 from http://www.medicalnewstoday.com/info/cancer-oncology/

Dai, Z.J., Lu, W.F, Gao, J., Kang, H.F., Ma, Y.G., Zhang, S.Q., et al. (2013). Anti-angiogenic effect of the total flavonoids in Scutellaria barbata D. Don. *BMC Complementary and Alternative Medicine*, 13:150. Retrieved from http://www.biomedcentral.com/1472-6882/13/150

Deryugina, E. & Quigley, J. (2009). Chapter two: Chick embryo

ISSN: 2094-5906

chorioallantoic membrane models to quantify angiogenesis induced by inflammatory and tumor cells or purified effector molecules. *Methods in Enzymology*, 4443, 21-41. DOI:10.1016/50076-6879.(08)02802-4

Dohle, D.S., Pasa, S.D., Gustmann, S., Laub, M., Wissler, J.H., Jennissen, H.P., et al. (2009). Chick ex ovo culture and ex ovo CAM assay: How it really works. *JoVE*, 33. DOI:10.3791/120

Eichhorn, M.E., Kleespies, A., Angele, M.K., Jauch, K.W., & Bruns, C.J., (2007). Angiogenesis in cancer: molecular mechanisms, clinical impact. *New Surgical Horizons*, 329: 371-379. DOI: 10.1007/500423-007-0150-0

Evers, E. (2014, March 17). Onion extract slow colon cancer growth just as chemodrug. *Rise earth [web log message]*. Retrieved from aftertheshift.blogspot.com/2014/03/ onion-extract-slows-colon-cancer-growth

Galeone, C., Giacosa, A., Franceschi, S., La Vecchia, C., Levi, F., Talamini, R., et al. (2006). Onion and garlic use and human cancer. *The American Journal of Clinical Nutrition*, 1027-1032. Retrieved April 12, 2014, from ajcn.nutrition.org

Garib Naseri, M.K., Yahyavi, H., & Arabian, M. (2008). Antispasmodic activity of onion (Allium cepa L.) peel extract on rat ileum. *Iranian Journal of Pharmaceutical Research*, 7 (2) , 155-159.

GMA News Online (2013). PHL has highest breast cancer rate in Asia. Retrieved November 4, 2015, from http://www.gmanetwork.com/news/story/329126/cbb/phl-has-highest-breast-cancer-rate-in-asia

Kabura, B.H, Musa, B. & Odo, P.E. (2008). Evaluation of the yield components and yield of onion (Allium cepa L.)-pepper (Capsicum annum L.) intercrop in the Sudan savanna. *Journal of Agronomy*, 7(1), 88-92.

Kubota, Y. (2011). Tumor Angiogenesis and Anti-angiogenic Therapy. *The Keio Journal of Medicine*, 61(2), 47–56.

Kumar, K.P., Bhowmik, D., Biswajit, Chiranjib, & Tiwari, P. (2010). Allium cepa: A traditional medicinal herb and its health benefits. *Journal of Chemical and Pharmaceutical Research*, 2(1), 283-291. Retrieved from www.jocpr.com

Kumar, S. & Pandey, A. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal*, 1-16. Retrieved from http://dx.doi.org/10/1155/2013/162750

Lachman, J., Pronek, D., Hejtmankova, A., Dudjak, J., Pivec, V., & Faitova, K. (2003). *Horticultural Science Journal*, 142-147. Retrieved from www.agriculturejournals.cz

ISSN: 2094-5906

Larger, E., Marre, M., Corvol, P., & Gasc, J.M. (2004). Hyperglycemia-induced defects in angiogenesis in the chicken chorioallantoic membrane model. *Diabetes*, 53, 752-761.

Lirdprapamongkol, K. , Kramb, J.P., Chokchaichamnankit, D., Srisomsap, C., Surarit, R., Sila-asna, M., Bunyaratvej, A., Dannhardt, G., & Svasti, J. (2008). Juice of Eclipta prostrate inhibits cell migration in vitro and exhibits anti-angiogenic activity in vivo. *In-vivo*, 22: 363-368.

Lokman, N.A., Elder, A.S.F., Ricciardelli, C., & Oehler, M.K. (2012). Chick chorioallantoic membrane assay as an in vivo model to study the effect of newly identified molecules on ovarian cancer invasion and metastasis. *International Journals of Molecular Sciences*, 13, 9959-9970. DOI:10.3390/IJMS13089959

Losso, J., Bagchi, D., & Shahidi, F. (2007). *Anti-angiogenic functional and medicinal foods*. US:CRC Press.

Melo-Reis, P.R., Andrade, L.S., Silva, C.B., Araujo, L.M.M., Pereira, M.S., Mrue, F., & Chen-Chen, L. (2010). Angiogenic activity of Synadenium umbellatum pax latex. *Brazilian Journal Biology*, 70(1), 189-194.

Murray, M. & Pizzorno, J. (2005). Chapter 10: The healing power of vegetables. *The Encyclopedia of Healing Foods*, 153-247. NY: Simon & Schuster, Inc.

Oklu, R., Walker, T. Wickey, S., & Hesketh, R., (2010). Angiogenesis and current antiangiogenic strategies for the treatment of cancer. *Emerging Technologies*, 21, 1791-1805. DOI: 10.1016/j-jvir.2010.08.009

Perez-Gregorio, R.M., Garcia-Falcon, M.S., Simal-Gandara, J., Rodriguez, A.S., & Almeida, D.P.F. (2009). Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest. *Journal of Food Composition and Analysis*, 23, 592-598. DOI:10.1016/j.jfca.2009.08.013

Pizzorno, L. & Murray, M. (2012). Allium cepa (onion). *Textbook of Natural Medicine, 4th edition*, 565-568. St. Louis, MO: Elsevier/Churchill Livingstone

Ribatti, D. (2010). The chick embryo chorioallantoic membrane as an in vivo assay to study antiangiogenesis. *Pharmaceuticals*, 3, 482-513. DOI:10.3390/ph3030482

Rudrappa, U. (2015). Retrieved March 1, 2015, from http://www.nutrition-and-you.com/ onion.html

Sahu, R. & Saxena, J. (2013). Screening of total phenolic and flavonoid content in conventional and non-conventional species of curcuma. *Journal of Pharmacology and Pythochemistry*, 2(1), 176-179. Retrieved from www.phytojournal.com

Scanlon, C.S., Inglehart, R.C. & D'Silva, N.J. (2013). Emerging value:

ISSN: 2094-5906

The chick chorioallantoic membrane (CAM) model in oral carcinogenesis research. *Carcinogenesis & Mutagenesis*, 1-3. DOI:10.4172/2157-2518.S5.005

Seyfi, P., Mostafaie, A., Mansouri, K., Arshadi, D., Mohammadi-Motlagh, H.R., and Kiani, A. (2010). In vitro and in vivo anti-angiogenesis effect of shallot (Allium ascalonicum): A heat-stable and flavonoid-rich fraction of shallot extract potently inhibits angiogenesis. *Toxicology in Vitro*, 24, 1655 – 1661. DOI:10.1016/j.tiv.2010.05.022

Setiawan, V.W., Yu, G.P., Lu, Q.Y., Lu, M.L., Yu, S.Z, Mu, L., et al. (2005). Allium vegetables and stomach cancer risk in China. *Asian Pacific Journal of Cancer Prevention*, 6, 387-395.

Silverstein, A., Silverstein, V. & Nunn, L. (2006). *Cancer*. Minnesota: Twenty-First Century Books.

Slimestad, R., Fossen, T., & Vagen, I.M. (2008). Onions: a source of unique dieatary flavonoids. *Jounal of Agricultural and Food Chemistry,* 55 (25) , 10067-80. DOI: 10.1021/jf0712503

Subramanian & Nagarajan (1969). Isolation and Identification of flavonoid "quercetin" from Citrullus cylocynthis (Linn.) Schrad. *Asian Journal Experimental Science*, 22(1), 137-142.

Thamilarashi, A.N., Mangalagowri, A., & Winkins, S. (2014). Anti-angiogenic activity of natural flavonoid myricetin on chick chorioallantoic membrane (cam) in-vivo. *International Journal of Pharmacy,* 4(3), 160-165. Retrieved from www.pharmascholars.com

World Cancer Research Fund International (2012). Retrieved October 11, 2014 from http://www.wcrf.org/int/cancer-facts-figures/worldwide-data

Yadav, S. & Kumar, P. (2012). Production, isolation and identification of flavonoids from aerial parts of Hiptage benghalensis. *International Journal of Life Science & Pharma Research*, 2(3). ISSN 2250-0480

ISSN: 2094-5906