European Journal of Molecular & Clinical Medicine
ISSN 2515-8260 Volume 07, Issue 02, 2020

In-Vitro Anti-Proliferative Effect Of Flavonoid Extract Of *Amaranthus Viridis* (Kolitis) Leaves Against MCF-7 Breast Adenocarcinoma Cell Line

Allysa Camille M. Malicdem[1], Michkaella Lorena O. Aducal[1], Jireh C. Cuasay[1], Denise Ansherina A. Dalisay[1], Alexander I. Mendoza[1], Glenda Marie E. Napiza[1], Carina R. Magbojos[1] and Oliver Shane R. Dumaoal[1]*

Lyceum of the Philippines (LPU), Batangas Campus,

P. Herera street corner Dona Aurelia street, Kumintang Ibaba, Batangas, 4200 Batangas, Philippines

Corresponding author: ordumaoal@lpubatangas.edu.ph

ABSTRACT

Cancer is one of the major concerns in health and medicine. Studies have been done by previous researches on the medicinal value of flavonoids present in *Amaranthus viridis*. However, limited claims were available in terms of their anti-proliferative ability against cancer cells. Flavonoid extracts were prepared by semi-purification using ethanol and ethyl acetate. Phytochemical screening using UV-Vis spectrophotometry was used to verify the presence of flavonoids. MTT cytotoxicity assay using MCF-7 breast cancer cells was employed to determine the cytotoxic effect of the plant extract. Four concentrations of the extract of *A. viridis* leaves were used as treatment to cultured cells. Doxorubicin and DMSO were used as the positive and negative control, respectively. An inhibition concentration (IC_{50}) of 18.33 ug/mL of the extract was reported after the assay, indicating that the administration of the treatment caused the death of the cells as seen when the yellow dye was not reduced into a purple formazan. Statistical analysis on the IC_{50} of doxorubicin and the *A. viridis* extract showed significant relationship, which indicated that doxorubicin has a more potent cytotoxic activity, though the extract had promising effects as seen in its IC_{50} being less than 30 ug/mL. These results can be attributed to the presence of the flavonoid quercetin which has been found out to possess cytotoxic effects against cancer cells. With these findings, a more natural, less toxic, cost-efficient and more readily available treatment regimen can be utilized for the management of cancer.

Keywords

Cancer; *Amaranthus viridis*; flavonoids; quercetin; MTT assay; cytotoxicity.

1. INTRODUCTION

The accumulation of altered genetic regulations which cause abnormal cell growth and expansion is the source of cancer [1]. Cancer continues to be a major threat in public health globally. In 2030, about 21.4 million new cases of cancer and 13.2 million deaths are expected to occur [2]. In the Philippines, cancer is the third most common cause of mortality, breast cancer being the most common. Apart from genetic mutations, epigenetic alteration also contributes to breast carcingenesis involving changes in DNA methylation (global hypomethylation and locus-specific hypermethylation), altered histone tail modifications patterns, and nucleosomal remodelling [1].

To date, breast cancer is still the leading cause of cancer among women and the second leading cause of mortality in the world with 11.6% of the total cases [3]. Despite the advances in diagnosis and treatment regimens for its management, the trend established over the past decade continues to show increasing incidence and mortality rates. At present, there is a low cancer prevention consciousness and survival rates since most cancer patients seek consultation only at advanced stages. One reason for treatment failure has been attributed to the development of resistance to the chemotherapeutic drugs used for therapy [4-5]. Another reason can be attributed to the financial burdens brought by cancer treatment. People, especially from the low- to middle-income families, do not have the capacity of undergoing treatment due to lack of resources which lead them to ignore their health and face the casualties brought about by their health problem.

The Philippines has an array of diverse plant resources. A number of researches were done on some traditional plants and were found out to exhibit therapeutic uses which are currently employed in the prevention and treatment of diseases. The desire to produce more effective but less toxic drugs fuels the continuous search for new bioactive compounds from plants [6]. Included in this rich biodiversity is *Amaranthus viridis* Linn.

Amaranthus viridis Linn. (family Amaranthaceae) known in the Philippines as Spinach Tagalog and Kolitis, and internationally known as the green amaranth, has been found out to be very useful in the field of medicine as it is traditionally used as anti-inflammatory, diuretic, analgesic, antiulcer, antiemetic and laxative agent [7]. Phytochemical screening done on *A. viridis* revealed the presence of biologically active components such as saponins, tannins and phenols, flavonoids, alkaloids, cardiac glycosides, steroids, triterpenoids and linoleic acid [8-9]. Studies showed that *A. viridis* leaves yield amounts of quercetin which is known to be an anti-proliferative agent [10]. In vitro work has concentrated on the direct and indirect actions of flavonoids on tumor cells, and has found a variety of anticancer effects such as cell growth and kinase activity inhibition, apoptosis induction, suppression of the secretion of matrix metalloproteinases and of tumor invasive behavior [11]. They were also known to have antiviral, antimicrobial, antihepatotoxic, antiosteoporotic, antiulcer, immunomodulatory, antiproliferative and apoptotic activity [12].

To date, no current studies are available regarding the potential of quercetin found in the leaves of *A. viridis* as an anti-proliferative agent in-vitro. Phytochemical screening of *A. viridis* using thin-layer chromatography has confirmed the presence of flavonoids and other phenolic compounds [13]. Hence, this study aimed to investigate and evaluate the anti-proliferative effect

of quercetin found in *A. viridis* leaves at different concentrations against MCF-7 breast cancer cell line by determining the inhibition concentration of the extract which can kill half of the cell population and to compare the resulting IC_{50} of the plant extract with that of doxorubicin which is a standard chemotherapeutic agent, and determine its significance. Through this study, a widely distributed plant with known therapeutic uses and will be utilized and studied further to reveal more of its medicinal value. Once proven effective, it can be utilized as a natural component for cancer treatment and management versus available synthetically made drugs.

2. MATERIAL and METHODS

2.1 Collection and Identification of Plant Specimen

A. viridis leaves were collected at Batangas City. The plant specimen was submitted for authentication by the Botany Division of the National Museum.

2.2 Preparation of Flavonoid Extract

The leaves were left to dry under a considerable amount of sunlight for 3 days. Thirty grams of leaves were finely grounded using mortar and pestle. The powdered leaves were defatted by soaking in 300 mL of cyclohexane for 24 hours. The defatting agent was removed after 24 hours using filtration. The powdered leaves were extracted with 99% ethanol by soaking for 24 hours. The mixture was filtered using Whatman (No. 1) filter paper. The filtrate was concentrated to dryness at 50°C by rotary evaporation [13]. The ethanolic extract was further extracted using 300 mL of ethyl acetate by soaking for another 24 hours. The mixture was filtered using Whatman (No. 1) filter paper. The filtrate was concentrated to dryness at 50°C by rotary evaporation [14].

2.3 Phytochemical Screening for Flavonoids

The standard (quercetin) and the plant extract were diluted using a two-fold serial dilution until the solution became clear. Quercetin (0.25 mg) at different dilutions (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256 and 1:512) was placed in separate test tubes. Water (1.25 mL) and sodium nitrate (0.75 µL) were added to each and were mixed. All the test tubes were kept in the dark place for 6 minutes. Then, 10% aluminum chloride (0.75 µL) was added into the test tube and kept for 5 minutes in the dark to complete the reaction. Finally, 5% sodium hydroxide (0.5 mL) and water (0.275 mL) were added to the test tube. The absorbance of all dilutions was measured at a fixed wavelength 510 nm using UV spectrophotometer. All values were plotted on a table and were analyzed using linear regression [15].

2.4 Cell Culture and Methyl-Thiol-Tetrazolium (MTT) Cytotoxicity Assay

The MTT cytotoxicity assay performed in this study was adopted from Mossman [16]. For the first day of the assay, MCF-7 cells from culture flasks were viewed using the inverted microscope to check the cells' viability. The cells adhering to the culture flasks were washed twice by phosphate buffered saline (PBS), pH 7.4. Trypsin was added to the cells from the culture flasks to facilitate the harvesting of the adherent cells. The flask was incubated at 37°C in a humidified 5% CO_2 atmosphere. A small amount was loaded in the hemocytometer for manual counting and to check the morphology of the cells. The cells were then seeded at 6×10^4 cells/mL in sterile 96-well microtiter plates separately. The plates were incubated overnight at 37°C and 5% CO_2.

For the second day of the assay which was the treatment phase, four concentrations of the extract were prepared as treatment: 50 ug/mL, 25 ug/mL, 12.5 ug/mL and 6.25 ug/mL, using the master dilution plate method. Doxorubicin was used as the positive control while dimethylsulfoxide (DMSO) as the negative control. Following incubation, cells were treated with 10 uL of each extract dilution. The treated cells were again incubated for 72 hours at 37°C and 5% CO_2.

After incubation, the media was removed and 20 uL MTT at 5 mg/mL PBS was added. The wells were further incubated at 37°C and 5% CO_2 for 4 hours after which, 150 uL DMSO was added to each cell. Absorbance was read at 570 nm. The Inhibition Concentration 50 (IC_{50}) was determined using the software "icpin" [17].

The reduction of tetrazolium salts has been widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT was reduced by metabolically active cell, through the action of mitochondrial dehydrogenase, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan was quantified using a microplate spectrometer (SLT Model Tecan Spectra III ELISA plate reader) at 570 nm. MTT was converted by viable cells into a purple formazan, indicating a negative reaction. Cytotoxic activity was observed when the yellow dye remained yellow at the end of the assay. The concentration at which cell proliferation was inhibited by 50% (IC_{50}) was computed by linear regression of absorbance against the concentrations tested.

2.5 Statistical Analysis

The absorbance and concentration of the quercetin standard and of *A. viridis* extract obtained from the phytochemical screening were plotted in a curve using linear regression. The Inhibition Concentration 50 (IC_{50}) was determined using the software "icpin". The relationship between the IC_{50} of the positive control and that of *A. viridis* extract was analyzed using independent sample T-test. The values of $p<0.05$ were considered significant. The computations were done using SPSS version 17.

3. RESULTS AND DISCUSSION

3.1 Plant Extract

In the preparation of the flavonoid extract, thirty grams of the dried leaves were soaked in 300 mL of ethanol and 300 mL of ethyl acetate, respectively. A total of 0.55 grams of sticky dark-green to black extract was obtained as seen in Figure 1. The total percent yield was 1.83%.

Figure 1. Plant extract obtained after rotary evaporation

3.2 Phytochemical Screening for Flavonoids using UV-Vis Spectrophotometry

Figure 2 showed the amount of quercetin equivalent of *A. viridis* extract. Phytochemical screening for flavonoids was carried out using quercetin as the standard to assess whether the specific flavonoid was present in the plant extract. As the absorbance decreased, the concentration of quercetin also decreased. Using linear regression, the curve was obtained and the quercetin equivalent of the extract was found out to be 110 mg/mL.

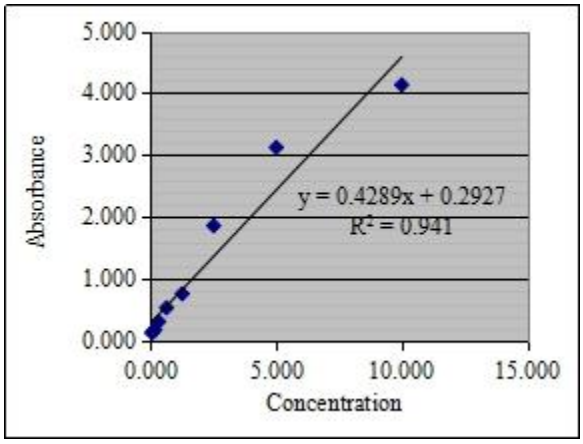

Figure 2. Quercetin Calibration Curve

The phytochemical screening revealed the presence of the said flavonoid. Having found out that *A. viridis* has known to contain quercetin, it can be deduced that the cytotoxicity conferred by the extract was due to the presence of the flavonoid.

Flavonoids and phenolic acids have protective role in carcinogenesis, inflammation, atherosclerosis, thrombosis and have high antioxidant capacity. Flavonoids interact with various enzymatic systems. Their inhibition of the enzymes, cyclooxygenase and lipooxygenase, resulted in a decrease of platelet activation and aggregation, protection against cardiovascular diseases, cancer chemoprevention and their anti-inflammatory activity [12]. Researches were conducted on the effect of quercetin against cancer cells in vitro. Quercetin has been observed to exert beneficial properties through different mechanisms such as inhibition of malignant cell growth by arresting them in the late G1 phase of the cycle, inhibition of pro inflammatory cytokine gene expression through the modulation of NF-kβ system and induction of apoptosis via direct activation of the mitochondria caspase cascade [18]. In the study conducted by Akbas et al. [19], results suggested that topotecan, a drug used as chemotherapeutic agent, has cytotoxic activity against the breast cancer cell lines in vitro. A combination with quercetin has increased the efficacy of topotecan in the treatment of breast cancers. The IC_{50} concentration of topotecan was 100 ng/ml in MCF-7 cell line and 160 ng/ml in MDAMB231 cell line. Treatment with quercetin has shown to enhance cytotoxicity of topotecan as 1.4-fold in MCF-7 and 1.3-fold in MDA-MB-231 cell line. This showed that synergism between topotecan and quercetin enhanced its cytotoxic effect against cancer cells. This demonstrated that quercetin enhanced the efficacy of doxorubicin treatment and, in turn, reduced its side effects, which were usually seen at higher concentrations [20].

In a study by Bojo et al. [17], quercetin has been found to be an anti-proliferative agent in a variety of mechanisms such as mutation in the p53 gene, inhibition of tyrosine kinase activity, induction of apoptosis and arresting cell growth at the G0/G1 phase of cell growth. In a study by Psahoulia et al. [21], quercetin and tumor necrosis factor–related apoptosis inducing ligand (TRAIL) has shown synergistic effect in inducing apoptosis in colon adenocarcinoma cell lines. TRAIL is one of the members of the tumor necrosis factor gene superfamily. It triggers apoptosis by binding as a homotrimeric subunit structure to death receptors DR4 (TRAIL-R1) and DR5 (TRAIL-R2), which contain a death domain in their cytoplasmic tail. TRAIL has gained interest as a potential candidate for cancer therapy mainly due to its ability to selectively induce apoptosis in many transformed cells but not in normal cells. The combination of quercetin and TRAIL caused reduction in cell viability, as supported by the measurement of the percentage of apoptotic nuclei as identified by Hoechst 33258 staining.

Yu et.al [22] stated in their study conducted that one of the antitumor effects of quercetin was to induce cytotoxic effects such as inhibition of cell proliferation and induction of apoptosis in human cancer cell lines such as breast, colon, leukemia, lung, and oral cancer cells. A parallel study on murine models by Lee et al. [23] showed that quercetin prevent carcinogenesis. It was reported that quercetin induced anti-proliferation by arresting G2/M phase and induction of caspase-dependent apoptosis in human promonocytic U937 cells.

In a study conducted by Lee et al. [24], quercetin activated AMPK in MCF breast cancer cell lines and HT-29 colon cancer cells, and this activation of AMPK seemed to be closely related to a decrease in cyclooxygenase-2 (COX-2) expression. AMPK (AMP-activated protein kinase) activation is known to regulate apoptosis in multiple cancer cells by a signaling pathway that included up-regulation of p53 and p21 proteins, activation of caspases, inhibition of molecules related to growth, and proliferation of cancer cells, such as COX-2. The application of a COX-2 inhibitor supported the idea that AMPK is an upstream signal of COX-2, and was required for the anti-proliferative and pro-apoptotic effects of quercetin.

3.3 MTT Cytotoxicity Assay

The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cytotoxicity assay was conducted at the Mammalian Cell Culture Laboratory and National Bioassays Office at the Institute of Biology, College of Sciences, University of the Philippines - Diliman. The cell culture used was MCF-7 human breast adenocarcinoma cell line.

As shown in Table 1, the mean absorbances of doxorubicin and *A. viridis* extract were compared. Doxorubicin at 50 ug/ml was not tested since it has been found out to have IC_{50} of less than 30 ug/ml, being used as a chemotherapeutic drug. Thus, testing above the said concentration will yield a negative result. The mean IC_{50} of doxorubicin was 1.9691, indicating that, doxorubicin is very potent against cancer cells. The mean IC_{50} of *A. viridis* extract was 18.3253, showing cytotoxic effect. The resulting IC_{50} was below the target concentration of 30 ug/mL, indicating that the extract was able to kill living cells. The result indicated that *A. viridis* extract had cytotoxic effects at a certain concentration which can be comparable to that of a standard drug.

Table 1. Comparison of the mean absorbance of each well per trial of the positive control and of *A. viridis* extract

Concentration (ug/ml)	**Doxorubicin**	***Amaranthus viridis* extract**
50	-	0.223
25	0.118	0.295
12.5	0.145	0.474
6.25	0.117	0.600
3.125	0.150	-
IC_{50}	**1.9691**	**18.3253**

A graphical representation of the comparison between the IC_{50} of doxorubicin and the plant extract carried out at three trials is shown in Figure 3. Among the three trials, the amount of Doxorubicin and plant extract were highest in trial 3.

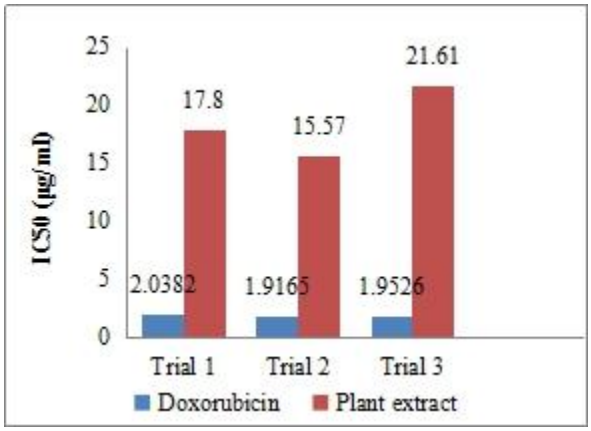

Figure 3. Comparison of the mean IC_{50} of doxorubicin and *A. viridis* extract on three trials

Plants have an almost unlimited capacity to produce substances that attract researchers in the quest for new and novel chemotherapeutics [25]. Plant flavonoids possess the propensity to modify or modulate the activities of a host of enzyme systems critically involved in cell surface signal transduction, immune function, cellular transformation, tumor growth, and metastasis [11]. *Amaranthus viridis*, which is a common weed and can be found even in backyards, showed promising application in pursuit of finding, if not better and efficient, possible alternatives in the treatment and management of cancer. Many researches had been conducted on *A. viridis* which emphasized on its anti-proliferative effect on different cancer cells in vitro. In the study conducted by Jin et al. [26], the ethyl ether fraction of *A. viridis* leaf extract showed significant inhibition of human colon cancer HT-29 cell growth in a dose-dependent manner by inducing G0/G1 phase arrest and apoptosis. Cell death induced by the fraction displayed characteristics of apoptosis and was associated with generation of reactive oxygen species and enhanced expression of caspase-3 and the Bax/Bcl-2 ratio in HT-29 cells.

Table 2 revealed the comparison of IC_{50} of the positive control and *A. viridis* extract. The obtained p-value was 0.011, less than 0.05 alpha level. This indicated that there is a significant difference on the IC_{50} values on the two tests. This showed that though the plant extract showed cytotoxic effect, it cannot be deduced to be better and a more efficient cure than the standard drug, doxorubicin. The extract is potent enough to kill the cancer cells, but it was not comparable with the effect brought about by doxorubicin being a standard chemotherapeutic agent.

Table 2. Comparison on the IC_{50} values of the positive control and *A. viridis* extract

	Group	**Mean**	**Mean Difference**	**95% Confidence Interval of the Difference**	**p-value**
IC_{50}	doxo	-16.36	-23.93	-8.78	*0.011
	Sample				

*Significant at *p-value < 0.05*

As shown in Figure 4, on the topmost left side of the wells, the positive control yielded a bright yellow color. On the top right side, the negative control showed a darkening of the wells. The bottom wells showed the color reactions yielded by the cells after treatment with *A. viridis* extract. As the concentration of the extract decreased, it can be noticed that the color produced darkens until it reached the point wherein the purple dye can no longer be converted into a yellow one. The last layer of cells showed a darkening of the wells but was not very comparable with the color intensity as observed in the negative control. The yellow color produced by doxorubicin indicated death of the cells. The darkening of the wells conferred by DMSO indicated the viability of the cells. Living cells were capable of reducing the yellow dye into a purple formazan, while dead cells cannot.

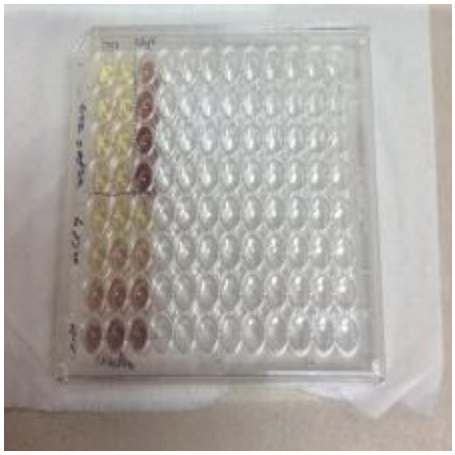

Figure 4. Color reactions of the positive control, negative control and the extract

Figure 5 showed the microscopic view of the cells after the treatment with *A. viridis* extract. Photo A showed the confluency of the cells after administration of the extract at 50 ug/mL. Photo B showed the aggregation of the cells after administration of the extract at 25 ug/mL. Photo C showed the flocculation of the cells after administration of the extract at 12.5 ug/mL.

Photo D showed the packing of cells after administration of the extract at 6.25 ug/mL. As the concentration of the extract decreased, it can be seen that lesser cells were killed and more cells were still viable, allowing the conversion of the yellow dye into a purple one. Such conversion was an indication of less cytotoxicity by the extract. This was seen when the testing of the extract at the lowest concentration of 6.25 ug/mL yielded a purple color of the well.

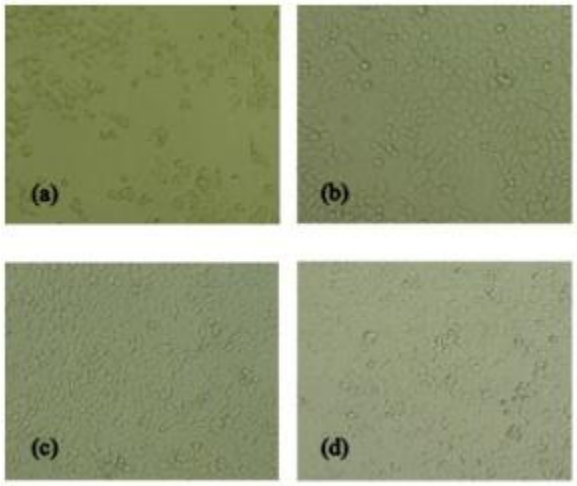

Figure 5. Appearance of MCF-7 cells after administration of the plant extract at 50 ug/ml (A), 25 ug/ml (B), 12.5 ug/ml (C) and 6.25 ug/ml (D)

Based on the results gathered, the plant extract showed activity in killing MCF-7 cells. This can be attributed to the mean value of the IC_{50} of the extract which was below the cut-off value of 30 μg/ml. According to the American National Cancer Institute (NCI), the criteria of cytotoxic activity for the crude extracts was $IC_{50} < 30$ μg/ml [25]. This standard was used as an indicator of significant cytotoxic activity in other studies focusing on the induction of cell death in cancer cells through the MTT cytotoxicity assay [27]. Any concentration above the said IC_{50} will give negative results in the assay, meaning the yellow dye will be converted into a purple dye. Conversion into a purple formazan was an indicator that cells were still viable. The IC_{50} of the extract on each trial was seen to be below the set concentration, therefore cytotoxicity occurred. Inhibitory activity was found out to be effective at 18.33 μg/ml.

4. CONCLUSION

Based from the results of the experimentation, quercetin found in the leaves of *Amaranthus viridis* is a potential chemotherapeutic agent with cytotoxic activity against breast cancer cells at a concentration of 18.33 ug/mL. The resulting IC_{50} which was below the standard of 30 ug/mL showed that the plant extract can kill viable cells. The yellow dye was not converted into a purple formazan indicating that a significant number of cells were killed. When compared to doxorubicin which is a standard chemotherapeutic agent used in the treatment for cancer, the extract showed promising cytotoxic effect though doxorubicin still proved to have a greater effect being the standard drug of choice

5. RECOMMENDATIONS

For the future researches, the group recommends the isolation of the specific flavonoid or other active component from *A. viridis*. Though the results had revealed the presence of the desired flavonoid, a more promising result can be possibly obtained if the specific component be tested. The researchers also suggested that other plant parts be utilized such as the stem or roots which were found to have possessed flavonoids and phenolic compounds that can be very promising in the treatment for cancer. Aside from in vitro testing, an in vivo study can also be done using laboratory animals however, toxicity tests should accompany. Aside from cytotoxicity as a vehicle of anti-proliferation, other tests can be included to support the claims that cell death was induced and that cell growth was inhibited. MTT cytotoxicity assay was just one of many ways on showing anti-proliferative effects on cancer cells. A comparison with normal cell lines can be made to further test if the plant extract is or not toxic to health living cells.

6. ACKNOWLEDGEMENT

This cytotoxicity assay was done at the Institute of Biology, University of the Philippines-Diliman. The method employed was based on the university's protocol. The researchers would like to acknowledge research assistants, especially Mr. Carlo Limbo from the Mammalian Cell Culture Lab, Institute of Biology, University of the Philippines, for their assistance in the performance of the assay.

7. REFERENCES

[1] Harahap, W. A., Sudji, I. R., & Nindrea, R. D. BRCA1 Promoter Methylation and Clinicopathological Characteristics in Sporadic Breast Cancer Patients in Indonesia. *Asian Pacific Journal of Cancer Prevention*, *19*(9), 2643-2649.

[2] American Cancer Society. (2012.) *Cancer Facts & Figures 2012*. Atlanta: American Cancer Society.

[3] Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal For Clinicians*, *68*(6), 394-424.

[4] Moreno-Aspitia, A., & Perez, E. A. (2009, June). Treatment options for breast cancer resistant to anthracycline and taxane. In *Mayo Clinic Proceedings* (Vol. 84, No. 6, pp. 533-545). Elsevier.

[5] Marquette, C., & Nabell, L. (2012). Chemotherapy-resistant metastatic breast cancer. *Current Treatment Options in Oncology*, *13*(2), 263-275.

[6] Olarte, E. I., Herrera, A. A., Villasenor, I. M., & Jacinto, S. D. (2013). In vitro antitumor properties of an isolate from leaves of Cassia alata L. *Asian Pac J Cancer Prev*, *14*(5), 3191-6.

[7] Ragasa, C. Y., Austria, J. P. M., Subosa, A. F., Torres, O. B., & Shen, C. C. (2015). Chemical constituents of Amaranthus viridis. *Chemistry of Natural Compounds*, *51*(1), 146-147.

[8] Reyad-ul-Ferdous, M., Shamim Shahjahan, D. M., Sharif, T., & Mohsina, M. (2015). Present biological status of potential medicinal plant of amaranthus viridis: a comprehensive review. *Am J Clin Exp Med*, *3*, 12-17.

[9] Iqbal, M. J., Hanif, S., Mahmood, Z., Anwar, F., & Jamil, A. (2012). Antioxidant and antimicrobial activities of Chowlai (Amaranthus viridis L.) leaf and seed extracts. *J. Med. Plants Res*, *6*(27), 4450-4455.

[10] Khan, M., Musharaf, S., Ibrar, M. & Hussain, F. (2010). Pharmacognostic evaluation of the *Amaranthus viridis L*. *Research In Pharmaceutical Biotechnology, 3 (1),* 11-16.

[11] Kanadaswami, C., Lee, L. T., Lee, P. P. H., Hwang, J. J., Ke, F. C., Huang, Y. T., & Lee, M. T. (2005). The antitumor activities of flavonoids. *In vivo*, *19*(5), 895-909.

[12] Plazonić, A., Bucar, F., Maleš, Ž., Mornar, A., Nigović, B., & Kujundžić, N. (2009). Identification and quantification of flavonoids and phenolic acids in burr parsley (Caucalis platycarpos L.), using high-performance liquid chromatography with diode array detection and electrospray ionization mass spectrometry. *Molecules*, *14*(7), 2466-2490.

[13] Patel, V., Sharma, V., & Patidar, A. (2014). Quantitative analysis of rutin and quercetin in Capparis spinosa and Brassica oleracea by HPLC. *Int J Pharm L Sci*, *5*(8), 3720-4.

[14] Deore, S. L., Nikole, K., Baviskar, B. A., & Khadabadi, S. S. (2013). Isolation and quantitative estimation of quercetin in Lagenaria siceraria fruit. *J Chromatograph Separat Techniq*, *4*(191), 2.

[15] Hossain, M. A., AL-Raqmi, K. A. S., AL-Mijizy, Z. H., Weli, A. M., & Al-Riyami, Q. (2013). Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. *Asian Pacific journal of tropical biomedicine*, *3*(9), 705-710.

[16] Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal Of Immunological Methods*, *65*(1-2), 55-63.

[17] Bojo, Z. P., Deaño, C. D., Jacinto, S. D., & Concepcion, G. P. (2010). Synergistic in vitro cytotoxicity of adociaquinone B and heptyl prodigiosin against MCF-7 breast cancer cell line. *Philipp Sci Lett*, *3*(2), 48-58.

[18] Blessing, E. O., & Francis, M. (2010). Dose–related effect of Quercetin in the human breast carcinoma MCF-7 cell line. *Am J Sci Ind Res*, *1*, 242-61.

[19] Akbas, S. H., Timur, M., & Ozben, T. (2005). The Effect of Quercetin on Topotecan Cytotoxicity in MCF-7 and MDA-MB 231 Human Breast Cancer Cells1. *Journal of Surgical Research*, *125*(1), 49-55.

[20] Atashpour, S., Fouladdel, S., Movahhed, T. K., Barzegar, E., Ghahremani, M. H., Ostad, S. N., & Azizi, E. (2015). Quercetin induces cell cycle arrest and apoptosis in CD133+ cancer stem cells of human colorectal HT29 cancer cell line and enhances anticancer effects of doxorubicin. *Iranian journal of basic medical sciences*, *18*(7), 635.

[21] Psahoulia, F. H., Drosopoulos, K. G., Doubravska, L., Andera, L., & Pintzas, A. (2007). Quercetin enhances TRAIL-mediated apoptosis in colon cancer cells by inducing the accumulation of death receptors in lipid rafts. *Molecular Cancer Therapeutics*, *6*(9), 2591-2599.

[22] Yu, C. S., Lai, K. C., Yang, J. S., Chiang, J. H., Lu, C. C., Wu, C. L., ... & Chung, J. G. (2010). Quercetin inhibited murine leukemia WEHI-3 cells in vivo and promoted immune response. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *24*(2), 163-168.

[23] Lee, J. H., Huh, C. H., Yoon, H. J., Cho, K. H., & Chung, J. H. (2006). Photoepilation Results of Axillary Hair in Dark-Skinned Patients by IPL: A Comparison Between Different Wavelength and Pulse Width. *Dermatologic Surgery*, *32*(2), 234-240.

[24] Lee, Y. K., Park, S. Y., Kim, Y. M., Lee, W. S., & Park, O. J. (2009). AMP kinase/cyclooxygenase-2 pathway regulates proliferation and apoptosis of cancer cells treated with quercetin. *Experimental & Molecular Medicine*, *41*(3), 201-207.

[25] Talib, W. H., & Mahasneh, A. M. (2010). Antiproliferative activity of plant extracts used against cancer in traditional medicine. *Scientia Pharmaceutica*, *78*(1), 33-46.

[26] Jin, Y. S., Li, C. M., Xuan, Y. H., Jin, Y. Z., Chen, M. L., & Row, K. H. (2013). Anticancer Activities of Extract from *Amaranthus viridis* L. *Asian Journal of Chemistry*, *25*(14).

[27] Jokhadze, M., Eristavi, L., Kutchukhidze, J., Chariot, A., Angenot, L., Tits, M., ... & Frédérich, M. (2007). In vitro cytotoxicity of some medicinal plants from Georgian Amaryllidaceae. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *21*(7), 622-624.