IN-VITRO CYTOTOXIC ACTIVITY OF GUYABANO (*Annona muricata)* PEEL ETHANOLIC EXTRACT ON COLON CANCER CELL LINE HCT-116

Alexander D. Cunanan, Niqui R. Decena,
Maejorie C. Orpilla, Casandra Kate I. Santiago

ABSTRACT

This research study evaluated the cytotoxic activity of Guyabano (*Annona muricata)* peel ethanolic extract against colon cancer cell line HCT-116 using MTT-assay. The phytochemical screening of Guyabano (*Annona muricata)* showed the presence of alkaloids, saponins, carbohydrates, flavonoids, proteins and phenols. The research study used Guyabano (*Annona muricata)* peel ethanolic extract as experimental group, Doxorubicin as the positive control and Dimethyl sulfoxide (DMSO) as the negative control. The results show that with the p-value at 0.000, there is a significant difference between the positive control, Doxorubicin against the experimental control, Guyabano ethanolic extract. Further, the Guyabano *(Annona muricata)* peel ethanolic extract computed percent inhibition from the absorbance readings were more than 100ug/ml but less than 1000ug/ml which indicates that it has weakly active cytotoxic activity against human colon cancer cell line HCT-116. Conclusion, Guyabano (*Annona muricata)* peel ethanolic extract has a weak potential in treating colon cancer.

Keywords: *Guyabano (Annona muricata), peel ethanolic extract, MTT assay, cytotoxic activity, human colon cancer cell line HCT-116*

INTRODUCTION

Cancer is the uncontrolled growth of abnormal cells anywhere in a body. These cells can infiltrate normal body tissues. Colon cancer is cancer of the large intestine (colon), which is the final part of the digestive tract (Davis and Baletine, 2017). According to American Cancer Society, Colorectal cancer is the third most common cancer diagnosed in both men and women in the United States. The American Cancer Society estimates that there are 97,220 new cases of colon cancer in the United States for 2018. Overall, the lifetime risk of developing colorectal cancer is: about 1 in 22 (4.49%) for men; and 1 in 24 (4.15%) for women.

Colon cancer has now overtaken liver cancer as the number one gastrointestinal cancer in the Philippines. There are more than 3000 new cases of Filipinos with colon cancer tolling about 2,000 deaths based only on reported cases (DOH, 2017). The most common factor of failure to undergo treatment of colon cancer is due to the cost of treatments and tests. Besides endoscopy and surgery to remove the tumors, colon cancer is detected and treated through standard

laboratory procedures and computed tomography (CT) colonography, CT scan for colon that will roughly cost at five (5) thousand dollars at least (Crosta et al, 2016).

Annona muricata, also known as Soursop, Guyabano (*Annona muricata*) in the Philippines is found mostly in warm tropical areas such as the Philippines and South America. Throughout history, each part of the graviola tree, such as the bark, leaves, roots, fruit, and seeds have been used for medicinal purposes. The seeds have been used to treat nausea and vomiting, while herbal medicine practitioners recommend using the fruit and leaves to relieve stomach distress, pain, cough, asthma, and fever. Guyabano (*Annona muricata*) has been used in many journals and researches, utilizing only the fruit and leaves. Guyabano (*Annona muricata*) also has been found out to contain acetogenins that can be used against several cancer cells like breast, lung and prostate cancers. (Manalastas, 2015)

From these bits of information, the researchers conducted a cytotoxic activity of Guyabano (*Annona muricata)* through in vitro analysis in HCT-116 colon cancer cells. This study only used the Guyabano (*Annona muricata*) Peel Ethanolic Extract (GPEE).

Research Questions

Generally, this research determined the in vitro cytotoxic activity of the Guyabano (*Annona muricata*) peel ethanolic extract in HCT-116 colon cancer cells.

Specifically, this research answered the following questions:

1. What are the phytochemical constituents present in the Guyabano (*Annona muricata*) peel ethanolic extract?
2. Is there a significant difference on the cytotoxic activity of the following treatment groups?
    a. Guyabano (*Annona muricata*) Peel Ethanolic Extract
    b. Positive control (Doxorubicin)
    c. Negative control (Dimethyl sulfoxide)

Hypothesis

There is no significant difference on the cytotoxic activity of the following treatment groups:

1. Guyabano (*Annona muricata*) Peel Ethanolic Extract
2. Positive control (Doxorubicin)
3. Negative control (Dimethyl sulfoxide)

Significance of the Study

The results of the study of In-vitro cytotoxic activity of the Guyabano *(Annona muricata)* peel Ethanolic extract is beneficial to the pharmacy students since it will be a basis and guide for those who wish to study similar case and research. It will be helpful to future researches about Guyabano (*Annona muricata*) extracts and its cytotoxic activity. This study shall be significant to the community since Guyabano (*Annona muricata*) is an indigenous plant that can be found anywhere, the people in a community will be aware that the Guyabano (*Annona muricata*) peeling extract can contribute to the colon cancer therapy. It will also help the farmers of Guyabano (*Annona muricata*) to motivatre them to produce more Guyabano (*Annona muricata*) plants.

Literature Review

Colon Cancer

The digestive system comprises of different organs that allow us to eat, chew, swallow any food and break it down to molecules which our body uses as a fuel to work and do its task. One major part of the digestive system is the colon or commonly called the large intestine. Colon is where the body extracts water and salts from the solid wastes, then the waste will then pass through the rectum and exits the body through the anus. Colon Cancer happens when tumorous growths develop in the large intestine (Crosta, 2017). It arises from the first layer of the colon lining which is the mucosa because these cells are more exposed to toxins from the food and bacteria, as well as they are more subjected to cell replacement because they are rapidly used over and over and mistakes in cell replacement would not be avoided which causes the uncontrolled growth of abnormal cells, that leads to the growth of polyps. Polyps are precancerous tumors that grow slowly over the years. As these polyps arise genetic mutations will unstable the cells. These precancerous tumors will then become cancerous and invades other layers of the colon lining (Stoppler, 2018). Colon cancer can be acquired by genetic factors, environmental exposures, lifestyle, and infectious diseases. The common symptoms are; rectal bleeding, unexplained weight lost, change in bowel habits and abdominal pain (Dragovich, 2018). It can be diagnosed by using the following test; Complete blood count, liver function tests, chest radiography, chest computed tomography, abdominal barium study. The colon and the rectum are the final portions of the tube that extends from the mouth to the anus. Food enters the mouth where it is chewed and then swallowed. The food that is not digested and absorbed enters the large intestine (colon) and finally the rectum. The large intestine acts primarily as a storage facility for waste. The large intestine is comprised of layers. The first is an inner layer of cells that line the cavity through which the undigested and digesting food travels, called the mucosa. The mucosa is attached to a thin second layer, the submucosa. The most common cancers of the large intestine (adenocarcinoma) arise from the mucosa, the inner layer of cells.

These cells are exposed to toxins from food and bacteria as well as mechanical wear and tear, and they are relatively turning over rapidly (dying off and being replaced). Mistakes (usually a series of mistakes involving genes within the replacement cells) lead to abnormal cells and uncontrolled proliferation of the abnormal cells that give rise to cancer.

There are different ways of staging cancer (Crosta, 2017). Stage 0, is the cancer in early stage and known as carcinoma. It only relies on the inner layer of the colon. Stage 1 cancer has grown into the next layer of the tissue or the submucosa of the colon but has not yet reached the lymph nodes or other organs. Stage 2 colon cancer has already reached the outer layer of the colon but has not spread yet beyond the colon. Step 3 colon cancer has grown through outer layers of the colon and it has reached one to three lymph nodes but not yet spread to distant sites. Stage 4 colon cancer has reached other tissues beyond the wall of the colon, as it progresses, the cancer reaches distant parts of the body. The development of cancer at each stage is not fixed but describes the phase at which certain developments take place.

Colon Cancer in the Philippines

In the Philippines, colon cancer is listed as the fourth most common cancer, according to the Philippine Cancer Society Registry. It comes after the three cancers, the breast, lung, and liver cancer in which the colon cancer is the only one which is not openly discussed (Tacio, 2017). Among of all cancers, colon cancer stands out as a disease which can be prevented but few believe that they would acquire the said cancer. And if it is discovered early, then the chance of prevention and treatment would be 100%, if the cancer already spread and metastasize, the 5-year rate of survival is low (Ruiz, 2017).

Conventional Management of Colon Cancer

In the Philippines, the awareness for colon cancer among Filipinos has been limited, as compared to lung and breast cancer (Tacio, 2017). Ruiz (2018) stated that the gold standard for colon and rectal cancer is colonoscopy as it can detect and remove early lesions like polyps. The procedure will involve a flexible fiber optic scope with a camera in which in it is inserted to the rectum to visualize the colon. Another invasive test is the Fecal Immunochemical Test (FIT) which is a stool test, is also a good screening alternative. Ruiz recommend a screening colonoscopy for persons between 50 to 75 years of age who are healthy, unless the risks of the procedure is high in that patient. If the person does not want to start with a colonoscopy, he suggest using the FIT. After a discussion with him, the patient can choose his preferred screening test. Cancer screening can save lives — this strategy has been shown to reduce CRC risk by as high as 70% — but not that many people are being screened. As an advocate .However, both are an invasive test and has the potential to cause complications like bleeding and puncturing the

colon. If the cancer is diagnosed early, it can be 80-95% curable. But surgery is the primary form of treatment, while radiation therapy may be likewise used along with chemotherapy before and after surgery (Gonzales, 2017).

Doxorubicin is chemotherapeutic drug called an anthracycline. It slows or stops the growth of cancer cells by blocking an enzyme called topo isomerase II. Cancer cells need this enzyme to divide and grow. Doxorubicin can intercalate the base pairs of the DNA's double helix thus damaging the DNA by binding to multiple molecular targets, a DNA-associated enzyme such as topoisomerase enzymes I and II and a range of cytotoxic effects occur in conjunction with anti-proliferation. The apoptosis pathway (a programmed cell death) is triggered when the attempt to repair the breaks in DNA fail and cellular growth is inhibited at phases G1 and G2. Doxorubicin is also known to inhibit both DNA and RNA polymerase, ultimately ceasing DNA replication and RNA transcription (Tacar, Dass, and Sriamornsak, 2012). The ability of Doxorubicin has been widely acknowledged for combating rapid dividing cells and slow disease progression. However, it induces apoptosis and necrosis in healthy tissue or noncancerous cells in the human body that causing toxicity in the brain, liver, kidney and heart.

Traditional and Alternative Medicine Act of 1997

Medicinal plants have been used in traditional healthcare systems since prehistoric times and are still the most important health care source for the most of the world's population (Sagnia et. al., 2014). The World Health Organization (WHO) has estimated that more than 75% of the world's total population depends on herbal drugs for their primary healthcare needs.

As more people resort to alternative health care, the emphasis on the exploitation of herbal plants in the country should be set in motion to attain cost-effective healthcare system. As an important source of nutrition and substances which produce physiological action on the human body, plants are recommended for their therapeutic values which can be used in drug development and synthesis (Ramawat and Merillon, 2008).

Republic Act No. 8423 also known as Traditional and Alternative Medicine Act (TAMA) of 1997 strives for the development and application of traditional and alternative medicine in the national health care delivery system. This act also promotes the importance of traditional medicine in the country as it has been embedded in our culture since the pre-Hispanic era. This act emphasizes scientific research, promotion, and advocacy on the use of traditional, alternative, preventive and curative health care modalities and formulation of standards, guidelines, and codes of ethical practice appropriate for the practice of traditional and alternative health care.

Ethnopharmacology of *Annona muricata Linn*

Guyabano (*Annona muricata*) (*Annona muricata L.)* also known as soursop, graviola, babana is a green, small tree about 5 to 7 meters high and weighs 2 to 5 kilos. The outer layer of the fruit is thin and the flesh is soft and white which have palatable flavor. Leaves are alternate, ovate, pointed, glabrous and shiny, 7 to 20 centimeters length. Flowers are broad and yellow green or yellow in color, consisting of six leathery petals in series. They have pointed tip and shaped like a heart. In the base of the flower is a cone-shaped which will form the fruit. It is found mainly in warm tropical areas such as Philippines and South America. There are two kinds of Guyabano (*Annona muricata*), the ordinary and the sweet. Both of them have identical description it is just that the other one is sweeter than the ordinary (Soheil, Mehran,& Kadir, 2015). Study found that the major phytochemical compounds are tannins, steroids and cardiac glycosides. It is traditionally used as sedative, a nerve tonic and used to maintain proper intestinal health. Anonaine and alkaloids anonaine present in the plant have been reported. Barks produce muricine and this is rich in hydrocyanic acid. Little amounts of these are found also in leaves and roots. Seeds produce toxins because of the oils present that can be irritating. Safe dose is not stated, but it is advised to take this under control and in minimal doses. Study also mentioned that pregnant woman is not allowed to take this plant because they might endure problems when they labor. Infusion of soursop leaves are not commended because there is a case of poisoning, it may damage our nervous system. Infusions are also contraindicated for patients with high or low blood pressure it may interact with action of cardiovascular drugs (Stuart, 2019).

Research Paradigm

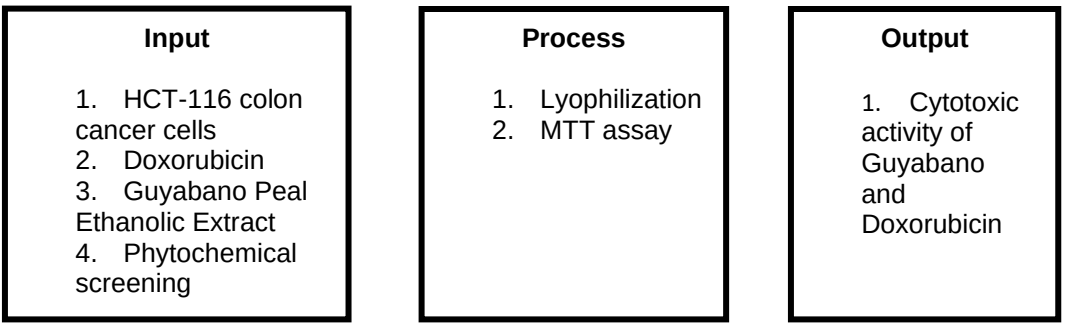

Figure 1. *Research Paradigm*

METHODS

Research Design

This study utilized the experimental method of research.

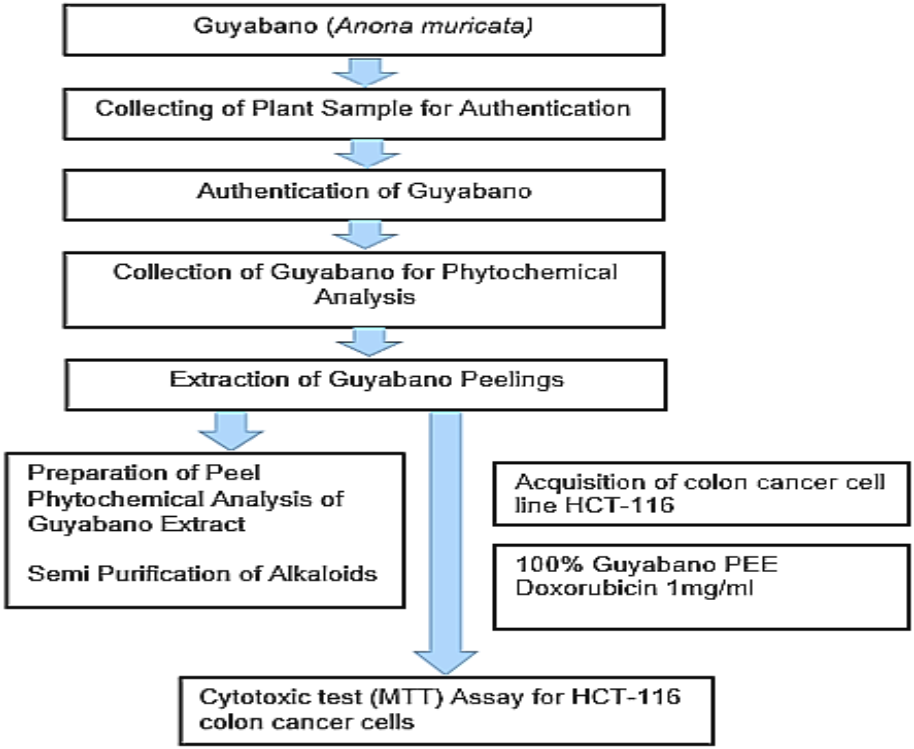

Figure 2. *Methodological Flowchart*

1. Collection and Preparation of Plant Sample

The fresh mature Guyabano (*Annona muricata)* fruits are collected at San Juan, Tuao, Cagayan. Authentication of the plant sample was done at Department of Agriculture. The plant was washed with running tap water to remove dirt and small insects. Then it was dried under the shade for 1 week.

2. Preparation of Peel Ethanolic Extracts

The peel was separated from the collected fruits. Three (3) kilograms of matured ripe peelings of *Annona muricata* fruits are collected. The dried peelings

were grinded and soaked in an 80% ethanol in an Erlenmeyer flask and sealed with a stopper for 2 weeks. After that, the ethanolic extract solution was filtered and placed in a rotary evaporator. Concentrations of 100% peel ethanolic extracts was prepared. The peel extracts was subjected to flame test to show that there is no ethanol left in the peel extract. The concentrated leaf extract was stored in a tightly closed container under a freezer at 20^{o}C.

3. Plant Authentication

The botanical verification and identification of the plant, *(Annona muricata)* was made and approved by Mr. Edward Yabis, Regional Manager of the Department of Agriculture- Regional Field Office II at Nursery Village, Tuguegarao, 3500 Cagayan.

4. Plant Extraction

Plant extraction process was based on the book of Guevarra (2005).

4.1. The powdered samples, weighing 100g, 50g, and 25g respectively for each sample was subjected to Soxhlet extraction individually with a sufficient amount of 80% ethanol. For the preparation of the solvent, the researchers added 80 ml of 95% from ethanol to a 95 ml of distilled water.
4.2. The temperature of the solvent was set to its boiling point (70^{o}C).
4.3. The extracts were collected and stored in different sterile Petri dish.
4.4. The excess solvent present in extracts were removed by oven drying for 10- 15 min at 50°C.

5. Phytochemical Screening

5.1. Phytochemical screening was conducted at Saint Louis University, Baguio City by a registered analyst. Results were turned over by the laboratory analyst at NSRU, Briege L. Martin, RMT.
5.2. Alkaloids, carbohydrates, glycosides, saponins, phytosterol, phenolic compounds, flavonoids and proteins were the constituents tested for the phytochemical analysis of the plant extract.

6. Lyophilization

The semi-purified plant extract was lyophilized at the University of the Philippines Los Baños, Laguna under the office of Philippine National Collection of Microorganisms, National Institute of Molecular Biology and Biotechnology before subjecting it to MTT Assay. Results were then turned over by the laboratory technician, Mr. Jovena Lanceras.

7. MTT Assay

7.1 Collection and Incubation of HCT-116 cell lines

The process of collection and incubation of breast cancer cell lines, HCT-116 was identified by the Institute of Biology of UP Diliman under Mammalian Cell Culture Laboratory.

7.2 Cytotoxicity assay

The MTT cytotoxicity assay performed in this study was adapted from Mosmann (1983). In detail:

7.2.1. Cells were seeded at 6 x 104 cells/mL in sterile 96-well microtiter plates. The plates were incubated overnight at 37°C and 5% CO2.

7.2.2. Eight two-fold dilutions of the sample were used as treatments starting from 100 µg/mL down to 0.78125 µg/mL. Doxorubicin had served as positive control while dimethyl sulfoxide (DMSO) had served as negative control.

7.2.3. Following incubation, cells were treated with each extract dilution. The treated cells were again incubated for 72 hours at 37°C and 5% CO2.

7.2.4. After incubation, the media was removed and 3-(4,5-dimethylethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye at 5 mg/mL PBS was added.

7.2.5. The cells were again incubated at 37°C and 5% CO2 for 4 hours.

7.2.6. After which, DMSO is used to dissolve the formazan crystals formed by the reduction of the dye by the live cells.

7.2.7. Absorbance was read at 570 nm.

7.2.8. The Inhibition Concentration 50 (IC50) was computed using GraphPad Prism 6. GraphPad Prism 6 computes for the IC50 of the sample by employing non-linear regression curve fit on the computed percent inhibition per concentration of the sample.

7.2.9. Samples with IC50 values less than 30 µg/mL are considered active (Jokhadze et al.,2007).

The figure is based on the research study of Bahuguna, Khan, Bajpai and Kang, MTT ASSAY to evaluate the cytotoxic potential of a drug. The figure shows how the assay was done basing on Mosmann's MTT assay protocol.

Data Analysis

The results gathered were tabulated and subjected to statistical treatment through the Statistical Package for Social Sciences (SPSS). To test the significant difference, the researchers used One-way ANOVA at 0.01 level of significance.

The table below shows the standard reading analysis of absorbance from spectrometers and colorimeters. According to the study of Bahuguna et. al, a decreased absorbance value suggests cytotoxicity. Absorbance values lower than the positive and negative control indicate a reduction in the rate of cell proliferation, while a higher absorbance value suggests increased cell proliferation.

Absorbance Reading Analysis

Reading	Qualitative Description
0.1-1.0nm	Useful absorbance
>1.0nm	Inaccurate/ Not useful absorbance
2nm	Outside meaningful range
3nm	Outside meaningful range

The table further shows the categories of determining the IC50 of an extract set by the U.S National Cancer Institute by its IC50. The lower IC50 value, the more it is active.

Criteria of cytotoxic activity established by the U.S National Cancer Institute by its IC50

IC50	Qualitative Description
≤ 20ug/mL	Very active
>20-100ug/ml	Moderately active
>100-1000ug/ml	Weakly active
>1000ug/ml	Inactive
*<4ug/ml	Potent

Applicable for pure compounds or drugs

Waste Disposal

All the materials that were used during the collection, preparation and extraction of plant extract were properly sterilized and properly disposed in coordination with the research adviser and with the personnel in-charge of the laboratory.

Disposal of the materials, extracts and cell cultures during the phytochemical screening, lyophilization and MTT Assay were taken by the proper authorities in charge in the facility of UP Diliman, UP Los Baños and Saint Louis University.

Ethical Considerations

In conducting the study, the researchers considered and followed the different ethical protocols involved in the study. The researchers asked permission from the proper authorities to conduct the study and obtained institutional ethics approval with a number of 117714. All references that were used in the study were

accurately credited for authorship. All institutions and persons involved in the conduct of the study were accurately credited as well. Since the study was conducted in-vitro, the researchers ensured that there was no physical and psychological harm to both humans and animals.

RESULTS

Table 1. *Organoleptic Test Result of Guyabano (Annona muricata) Peel Ethanolic Extract*

Extract	Observation
Appearance	Turbid
Color	Greenish to brownish liquid
Smell	Strong, Agreeable odor

Table 1 shows that the appearance of the peel ethanolic extract of Guyabano (*Annona muricata*) has a yellow, creamy color and has a strong sweet odor.

Table 2. *Phytochemical Screening Result*

Chemical Constituents	Results
Alkaloids	+
Carbohydrates	+
Glycosides	-
Saponins	+
Tannins	-
Phenols	+
Flavonoids	+
Proteins	+
Phytosterol	-

Legend: Presence (+), Absence (-)

Table 2 shows that alkaloids, carbohydrates, saponins, phenols, flavonoids and proteins are present in the extract while tannins and glycosides are not present.

Table 3a. *Absorbance Reading on the Cytotoxic Activities of all Treatment Groups*

Group	Mean	df	F-value	p - value	Decision
Negative	1.1122	2	65.485	0.000	**Reject Ho**
Positive	0.4643				
Extract	1.1364				

Table 3a shows that with a p-value of 0.000, there is a significant difference on the cytotoxic activity of the 3 treatment groups.

Table 3b. *Post Hoc Test Analysis on the Absorbance Reading of the Cytotoxic activities of all Treatment Groups*

Group	Mean	Negative	Positive	Extract
Negative	1.1122	1		
Positive	0.4643	0.000*	1	
Extract	1.1364	0.717	0.000*	1

Table 3b shows that there is a significant difference on the cytotoxic activity on the positive group against the extract and the negative group.

Table 4a. *Absorbance Reading of the Cytotoxic Activities of the Treatment Groups, when grouped according to Trial 1*

Group	Mean	df	F-value	p - value	Decision
Negative	0.7968	2	62.089	0.000	**Reject Ho**
Positive	0.3288				
Extract	0.8258				

Table 4a shows that at trial 1 with a p-value of 0.000, there is a significant difference on the cytotoxic activity of the 3 treatment groups.

Table 4b. *Post Hoc Test Analysis on the Absorbance Reading of the Cytotoxic activities of treatment groups, when grouped according to Trial 1*

Group	Mean	Negative	Positive	Extract
Negative	0.7968	1		
Positive	0.3288	0.000*	1	
Extract	0.8258	0.565	0.000*	1

Table 4b shows that at trial 1, with a p-value of 0.000, there is a significant difference on the cytotoxicity activity between the positive group against the extract and the negative group.

Table 5a. *Absorbance Reading of the Cytotoxic Activities of the Treatment Groups, when grouped according to Trial 2*

Group	Mean	df	F-value	p - value	Decision
Negative	1.0532	2	83.944	0.000	**Reject Ho**
Positive	0.3825				
Extract	1.0677				

Table 5a shows that at trial 2, with a p-value of 0.000, there is a significant difference on the cytotoxic activity of the 3 treatment groups.

Table 5b. *Post Hoc Test Analysis on the Absorbance Reading of the Cytotoxic activities of treatment groups, when grouped according to Trial 2*

Group	Mean	Negative	Positive	Extract
Negative	1.0532	1		
Positive	0.3825	0.000*	1	
Extract	1.0677	0.811	0.000*	1

Table 5b shows that at trial 2, with a p-value of 0.000, there is a significant difference on the cytotoxicity activity between the positive group against the extract and the negative group.

Table 6a. *Absorbance Reading of the Cytotoxic Activities of the Treatment Groups, when grouped according to Trial 3*

Group	Mean	df	F-value	p - value	Decision
Negative	1.4867	2	42.028	0.000	**Reject Ho**
Positive	0.6814				
Extract	1.5157				

Table 6a shows that at trial 3, with a p-value of 0.000, there is a significant difference on the cytotoxic activity of the 3 treatment groups.

Table 6b. *Post Hoc Test Analysis on the Absorbance Reading of the Cytotoxic activities of treatment groups, when grouped according to Trial 3*

Group	Mean	Negative	Positive	Extract
Negative	1.4867	1		
Positive	0.6814	0.000*	1	
Extract	1.5157	0.780	0.000*	1

Table 6b shows that at trial 3, with a p-value of 0.000, there is a significant difference on the cytotoxicity activity between the positive group against the extract and the negative group.

Table 7. *Percent Inhibition Computed from the Absorbance Readings of each Concentration of the Sample (Annona muricata) against HCT-116 cells.*

Conc (µg/mL)	***Annona muricate***					
	Trial 1		**Trial 2**		**Trial 3**	
100	4.0192	-4.1800	-3.0831	-9.4822	-12.3710	-7.87211
50	0	-6.5	-0.9681	3.55002	-1.42357	3.01819
25	0.2509	0.87829	-5.7745	-1.9248	-0.90589	-1.73324
12.5	-1.5700	-10.266	13.0591	-8.5404	-4.42616	-2.06966
6.25	-0.6769	-13.476	5.41872	0.85087	1.33389	-1.64167
3.125	-1.8769	-3.3368	-14.697	2.8377	1.44270	-1.57077
1.5625	-0.5411	-4.5099	-5.0768	-1.5370	-2.48050	0.02837

0.78125	-7.1232	-7.1232	4.54297	-2.0251	-1.59761	-2.61120
IC_{50}	**>100 µg/mL**		**>100 µg/mL**		**>100 µg/mL**	

Table 7 shows that the IC_{50} of the experimental control, ethanolic peel extract of *Annona muricata*, is found to be higher than >100 µg/mL. Based on the criteria given for IC_{50} by the U.S. National Cancer Institute, it is considered as weakly active in exhibiting a cytotoxic activity against the human colorectal cancer cell line HCT-116.

DISCUSSION

The phytochemical analysis of guyabano peel showed that it contains the following secondary metabolites: alkaloids, carbohydrates, phenolic, saponins, flavonoids and proteins. There are two hundred and twelve known bioactive compounds found in *A. muricata.* The most dominant compounds found are acetogenins, followed by alkaloids, phenols and other compounds (Coria-Tellez et al 2015). The alkaloids found could induce the cytotoxic activity of *A. muricata* (Matsushige et al., 2012). The phenolic compounds are considered as the major phytochemical responsible for the antioxidant activity of the plant (George et al., 2014).

The results from this study showed Guyabano peel ethanolic extract with an IC50 of more than 100ug/ml at trial 1, 2 and 3. This study will contribute to the handful of studies reported using *A. muricata* peel ethanolic extract against human colorectal cancer cell line HCT-116. Nawwar et al. (2012) reported that 1.6 µg/ml and 50 µg/ml from hydroalcoholic extract of A. muricata leaves increased the viability of non-cancerous cells while 100 µg/ml did not alter their viability. While this study used the peel of the guyabano, the leaves are potential to have a cytotoxic activity to HCT-116 with IC50 of 12.25ug/ml, 3.91ug/ml, and more than 100ug/ml on n-hexane, ethyl acetate and methanol extract, respectively (Moghadamtousi et al., 2015). A study has also shown that among all solvent used in extraction, organic solvents, ethanolic and pentatonic were the most active *A. muricata* extracts against cancer cell in-vitro growth (Menan et al., 2006). Regardless of these studies, only one published research has utilized the A. muricata pericarp against cell line U-937.

The study of Jaramillo et al (2000) found three (3) acetogenins; namely: annonacin, annonacin A and annomuricin A. While this study mostly focused on the anti-leshmanial activity of the plant, the pericarp of A. muricata was also tested on cell line U-937, a lymphoma or cancer of white blood cell lymphocytes. While the study of Jaramillo showed a cytotoxic activity, it has a downside of using pericarp, a collective term of the peels of plant, which coincides with this study. Even though this research conducted showed a weekly active cytotoxic activity, the negative

result may be due to the fact that *A. muricata* peel is not traditionally used in treating cancer.

CONCLUSION

The Guyabano peel ethanolic extract has weak cytotoxic activity against human colon cancer cell line HCT-116.

RECOMMENDATIONS

Based on the results of this study, the following recommendations were made:

1. Conduct a semipurification and isolation of plant.
2. Use other parts of plants that have not yet been extensively researched or published.
3. Conduct a similar study in different cell line.
4. Modify the method by using different but tested solvent extraction.

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