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Anti-cancer activity of *Carica papaya* leaf ethanolic extract and fractions against selected human cancer cell lines

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Abstract

The anti-cancer activity of *Carica papaya* leaf ethanolic extract and ethyl acetate fraction were evaluated against selected human cancer cell lines by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The leaf ethanolic extract of *C. papaya* was subjected to fractionation by using hexane, chloroform and ethyl acetate to separate compounds based on their polarity. The ethanol and ethyl acetate fraction of the plant were tested for their cytotoxic and anti-cancer activity against HCT- 116 human colon cancer cell line; mcF7 – human breast cancer cell line and A549-Human adenocarcinoma cell line by using MTT assay. There were eight three-fold dilutions of the plant samples were used as treatments starting from 100µg/mL down to 0.05µg/mL. Results showed that the ethanol extract and ethyl acetate fraction of *C. papaya* exhibited cytotoxic effect against HCT 116- Human colon cancer cell lines having IC_{50} values of 24.42 µg/ml and 34.87 µg/ml, respectively. On the otherhand, ethyl acetate fraction of the said plant manifested low cytotoxic activity againstmcF7 – Human breast cancer cell line and A549-Human adenocarcinoma cell line having IC_{50}>100 µg/ml of the two cell lines. Based on the results, the ethanolic extract and ethyl acetate fraction of *C. papaya* was established to significantly reduced HCT 116- Human colon cancer cell line proliferation, manifesting the cytotoxic/bioactive compounds in the said plant leaves and very promising sources for the development of anti-cancer agents.

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Introduction

Cancer is a major public health threat worldwide which significantly affect the domestic development of the country. In the Philippines, cancer statistics considered it as the top 4 leading diseases causing the death of both adults and children individuals. Cancer is characterized by the following properties: uncontrolled cell division; abnormal cell growth; lethal/deadly to its neighboring tissue; others produce minor tumor growth; and abnormal cytoskeletal proteins (Lamorte 2014). The World Health Organization (2015) believed that cancer is one of the main causes of death of human population in which 8.2 million mortality out of 14 million cases in 2012. In addition, cancer is thought to be originated by the genetic and environmental factors. Treatment for cancer usually involved chemotherapeutic drugs which work by inactivating cell division, specially fast-dividing cells (Thenmozhi *et al.*, 2011).

Nature provides us with various plants which provide food and phytomedicine (Dawara *et al.*, 2012). Humans have been greatly benefitted by plants, one of which is the medicinal sources it can provide (Rojas *et al.,* 2006). Long before the advent of drugs, ancestors had sought cure basically from plants. Although the idea of plants for medicine may seem primitive, it should not be underestimated. There have been lots of plants that are used as a basis for synthesizing and formulating drugs for treating different kinds of disease (Dias *et al.,* 2012).

It is estimated that eighty percent (80%) of the global population hang on to customary medication which utilized plant products for the treatment and cure of different diseases. The role of medicinal plants is very significant in the development of new drugs (WHO, 2002). Lotufo *et al.* (2005) stated that many drugs that are presently used chemotherapeutic agents were isolated from various types plants. Schimtt (2007) stated that plants that inhibit cell division belong to the effective chemotherapeutic drugs that are used for anti-cancer treatment.

Natural products or phytochemicals which are known to possess medicinal value includes steroids, tannins, alkaloids, flavonoids and saponins. Bandaranayake (2002) stated that tanins play significant role in various physiological events such as host mediated tumor activity, and stimulation of phagocytic cells in humans. In addition, tannins have anticancer activity and can be used in cancer prevention (Li and Wang, 2003) and antimicrobial property (Hayek *et al.*, 2013). Alkaloids are strong potential for the elimination and reduction of human cancer cell lines. Isolated plant alkaloids and their synthetic derivatives are used for their analgesic, anti-spasmodiac and bactericidal effects (Okwu, 2004). Moreover, saponins are cancer protective agents acting as antioxidants, and antimutagens (Nobori *et al.*, 1994). Terpenoids show cytotoxicity against various tumor cells, anti-cancer efficacy and cancer defensiveness (Thoppil and Bishayee, 2011). Glycosides exhibit anticancer activities throughout various phases of carcinogenesis. These include antiproliferative, proapoptotic, and chemotherapy sensitization effects (Kumar 2013).

Cancer cell lines derived from human tissue samples are essential models used to study and test therapeutic potentials of various anti-cancer substances (Sharma *et al.*, 2010). At present, various cell lines have been cultured both in vitro and in vivo as monolayer cultures and xenografts in ice, respectively (Mattern *et al.*, 1988).

Cancer cell lines have been successfully utilized in different research undertakings and manifest a very good model in cancer related studies (Louzada *et al.*, 2012). In genetic approach, cancer cell lines provide substantial information especially on the deregulated genes and signaling pathways in cancer disease (Vargo-Gogola and Rosen, 2007). Moreover, the development and testing of chemotherapeutic drugs that are presently used originated through the use of cell models (Louzada *et al.*, 2012; Nakatsu *et al.*, 2005). The use of the suitable in vitro model such as cell lines in cancer studies is very significant for the study of genetic and cellular pathways (Louzada *et al.*, 2012).

Carica papaya, locally known as papaya is recognized for its folk medicinal uses.

In fact, almost all parts of the plant such as roots, leaves, flowers, seeds, bark and even latex have been widely used for medicinal purposes. The ripe fruit aside from being nutritious, it is also utilized as topical ulcer dressing for faster healing of chronic skin ulcers (Hewitt *et al.*, 2000). Dhanamani *et al.* (2013) stated that the green fruit is utilized for different human and animal diseases such intestinal helminthiasis, diabetes mellitus, malaria, hypertension and jaundice. In India, the leaves papaya is commonly used to control asthma and fever (Doughari *et al.*, 2007). In addition, it is also used as traditional herbal treatment for cancer in Vietnam and Australia (Otsuki *et al.*, 2010).

Various scientific researchers have been conducted to confirm the traditional medicinal uses of *C. papaya*. Nguyen (2016) revealed that *C. papaya* possesses anthelminthic, anti-protozoan, antibacterial, antifungal, anti-viral, anti-inflammatory, anti-hypertensive, hypoglycaemic and hypolipidaemic, wound healing, free radical scavenging, anti-sickling, neuroprotective, diuretic, abortifacient and antifertility properties. Studies conducted of this plant species focused only on antimicrobial and antioxidant activity. In addition, previous studies conducted on antimitotic and anti-oxidant of *C. papaya* leaves are limited only on the crude extract. Another study conducted on anti-tumor activity of aqueous leaf extract of *C. papaya* showed inhibition of tumor cell growth (Otsuki, 2010). In addition, a study conducted by Gutierrez (2016) showed antimitotic effect of crude ethanolic of *C. papaya* against sea urchin embryos. The inhibition of cell division in sea urchin embryos is concentration-dependent. This study aims to determine the cytotoxic and anti-cancer properties of *Carica papaya* leaf ethanolic extract and ethyl acetate fraction.

Materials and methods

Preparation and Extraction of Plant Samples

Carica papaya leaves were collected in the rural barangay of Consolacion, Cebu, Philippines. The collected plant samples were washed with tap water and rinsed with distilled water. It was air-dried at room temperature. After drying, the leaf samples were homogenized using a blender.

Leaf samples were macerated with 100% ethanol (analytical grade) for seventy-two hours. The soluble ethanolic extract was filtered and the filtrate was concentrated in vacuo at 40°C using a rotary evaporator yielding a concentrated ethanolic crude extract which was used for solvent partitioning and brine shrimp lethal toxicity assay.

Solvent Fractionation

Ethanolic extract was exposed to solvent fractionation using hexane, chloroform and ethyl acetate in order to separate the natural products from the crude ethanolic extract based on their polarity. Three hundred (300) mL of the concentrate (semi-liquid) crude ethanolic extract was placed in a separatory funnel. Equivalent amount (300mL) of 95% *n*-hexane was mixed. Allow the solution to separate into two layers for 24 hours; the upper layer contains the compounds soluble in *n*-hexane. Repeat the same procedure until the upper layer becomes colorless showing that all of the hexane fraction was separated. The fraction was concentrated through rotary evaporation.

The bottom layer of the hexane fraction was collected and subjected to chloroform fractionation. Mix the bottom collected extract with equal amount of chloroform in a 1000mL separatory funnel then add equal amount of distilled water. Allow the mixture to separate in an hour. The upper layer containing the chloroform fraction was collected and concentrated to recover the chloroform extract. After chloroform fractionation, the aqueous layer was collected and exposed to ethyl acetate fractionation. The aqueous layer was mixed with ethyl acetate in a separatory funnel and 300mL of distilled water be added. The mixture was allowed to separate and form two layers. The upper layer containing the ethyl acetate was collected and concentrated using rotary evaporator.

Methyl thiol tetrazolium (MTT) cytotoxicity assay

The MTT cytotoxicity assay performed in this study was adapted from Mosmann (1983 using three (3) selected human cancer cell lines, HCT- 116 human colon cancer cells, mcF7 – human breast cancer cell line and A549-Human adenocarcinoma cell line which was conducted at the Mammalian Cell Culture

Laboratory (MCCL), Institute of Biology, College of Science, University of the Philippines Diliman, Quezon City, Philippines. In detail, cells were seeded at 4 or 6 x 10^4 cells/mL (depending on the cell culture used) in sterile 96-well microtiter plates. The plates were incubated overnight at 37°C and 5% CO_2. Eight three-fold dilutions of the sample were used as treatments starting from 100µg/mL down to 0.05µg/mL. Doxorubicin served as positive control while dimethyl sulfoxide (DMSO) served as negative control. Following incubation, cells were treated with 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 3-(4,5-dimethylethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye at 5mg/mL PBS was added. The cells were again incubated at 37°C and 5% CO_2 for 4 hours. After which, DMSO is used to dissolve the formazan crystals formed by the reduction of the dye by the live cells. Absorbance was read at 570 nm.

Statistical Analysis

Data are presented as mean. The Inhibition Concentration 50 (IC_{50}) of the plant extract and fraction against selected human cancer cell lines was computed using Graph Pad Prism 6. GraphPad Prism 6 computes for the IC_{50} of the sample by employing non-linear regression curve fit on the computed percent inhibition per concentration of the sample.

Results and discussion

Table 1 shows the in vitro cytotoxicity of *C. papaya* ethanolic extract and ethyl acetate fractions against three selected human cancer cell lines: HCT116-Human colon cancer cell line,mcF7 – human breast cancer cell line and A549-Human adenocarcinoma cell line. Results showed that the ethanolic extract and ethyl acetate fraction of *C. papaya* exhibited cytotoxic effects against HCT 116- Human colon cancer cell lines having IC_{50} values of 24.42 µg/ml and 34.87 µg/ml, respectively. On the otherhand, ethyl acetate fraction of the said plant manifested low cytotoxic activity againstmc F7 – Human breast cancer cell line and A549-Human adenocarcinoma cell line having IC_{50}>100 µg/ml of the two cell lines.

Fig. 1 shows the percent inhibition from the absorbance readings of the ethanol extract and ethyl acetate fractions of *C. papaya* against HCT116-Human colon cancer cell lines. It is noted that the percentage of inhibition of the proliferation of cancer cells was found directly proportional to the concentration of *C. papaya* ethanolic extract and ethyl acetate fraction at 20 µg/ml onwards. In other words, cytotoxic activity increased gradually with the increase in concentration of the test plant sample.

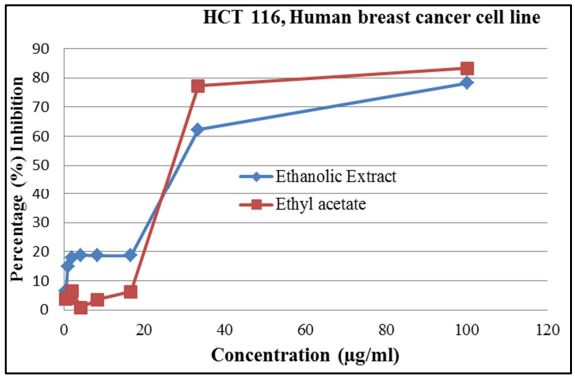

Fig. 1. Percent inhibition from the absorbance readings of the ethanolic extract and ethyl acetate fractions of *C. papaya* against HCT116-Human colon cancer cell line.

MTT assay was conducted to determine the viability of *C. papaya* as a potential anti-cancer agent against selected human cancer cell lines. Table 1 shows the inhibition concentration, IC_{50} of *C. papaya* leaf ethyl acetate fractions against three selected human cancer cell lines. Results show that the ethanolic extract and ethyl acetate fraction of the tested plant manifest cytotoxic effect against HCT116-human colon cancer cell line. On the other hand, the said plant showed low biological activity againstmcF7-human breast cancer cell line and A549-human adenocarcinoma.

Phytochemical studies conducted in *C. papaya* leaves revealed the presence of alkaloids, flavonoids, glycosides, saponins and tannins (Alorkpa *et al.*, 2016). Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity, potential in the reduction and elimination of human cancer cell lines, and the

extreme presence of these in plants can cause poisoning (Nobori *et al.*, 1994). It is also successfully developed into chemotherapeutic drugs, such as camptothecin (CPT), a famous topoisomerase I (TopI) inhibitor , and vinblastine, which interacts with tubulin (Li *et al.*, 2007).

Table 1. Inhibition concentration, IC_{50} of *C. papaya* leaf ethanolic extract and ethyl acetate fractions against three selected human cancer cell lines.

Plant	Ethyl acetate Fraction			Ethanol Extract
	Cell line IC_{50} (µg/ml)			
	HCT116-Human colon cancer cell line	MCF7 – Human breast cancer cell line	A549-Human adenocarcinoma cell line	HCT116-Human colon cancer cell line
Carica papaya	34.87	>100	>100	24.42
Doxorubicin	0.48	1.198	0.4776	0.48

Some well-developed semi-synthetic anti-cancer drugs are alkaloid derivatives including vinblastine, vinorelbine, vincristine, and vindesine. They are the most important active ingredients in traditional medicine and have been approved for cancer treatment in the United States and Europe (Moudi *et al.*, 2013). Flavonoids serve as a health promoting compound as a results of its anion radicals (Hausteen 1983). Moreover, Lotufo (2003) stated that flavonoids can inhibit the proliferation of cell lines and demonstrated strong cytotoxicity towards colon cancer cells. It has also the capability to inhibit the proliferation of cell lines and demonstrated strong cytotoxicity towards colon cancer cells (Ahmed *et al.*, 2015) and interfere with cyclin-dependent cell cycle regulation and interact with drug transport (Halliwel and Gutteridge 2007). Flavonoids exert a wide variety of anticancer effects: they modulate ROS-scavenging enzyme activities, participate in arresting the cell cycle, induce apoptosis, autophagy, and suppress cancer cell proliferation and invasiveness (Yahfoufi *et al.*, 2018). Glycosides exhibited in vitro cytotoxic and cytostatic effects against various human cancer cell lines, which is attributable to their ability to induce cell-type-specific cell death modalities (Cerella *et al.*, 2013). Saponins are known to produce inhibitory effect on inflammation and they are major ingredients in traditional Chinese medicine and thus responsible for most of the observed biological effects (Liu and Henkal, 2002). It demonstrates significant anticancer activity, such as anti-proliferation; anti-metastasis; anti-angiogenesis (Zeng *et al.*, 2015) and reduces the side-effects of radiotherapy and chemotherapy (Zhao *et al.*, 2016). Tannins have anticancer activity and can be used in cancer prevention (Li and Wang, 2003) and also significant in the stimulation of phagocytic cells, host mediated tumor activity and anti-infective actions in humans (Bandaranayake, 2002).

C. papaya significantly reduced the cell viability of selected human cancer cell lines in a concentration-dependent way. The inhibibiton of cell proliferation induced by the ethanolic extract and ethyl acetate fraction could be due to the induction of cell death which provides evidence for the *in vitro* cytotoxic activity. Moreover, the significant inhibition of cancer cell proliferation manifested by the ethyl acetate fraction of *C. papapya* could be attributed to the intensive capability to the said solvent to extract bioactive compounds which is supported by previous studies conducted by Waheed *et al.* (2014) which extracted various phytochemicals such as alkaloids, cardiac glycosides, flavonoids, phenolics, saponins, carbohydrates, terpenoids, tannins *Ballota limbata* (Lamiaceae) using ethyl acetate.

Furthermore, there is selective cytotoxicity and differences in the sensitivity of the specific cancer cell line to the ethyl acetate fraction of *C. papaya* which propose that the bioactive compounds present in the tested plant may react only in drug-sensitive cell lines such as HCT116-human colon cancer cell line but not inmcF7-human breast cancer and A549-human adenocarcinoma cell lines.

It is recommended that cytotoxic studies on other parts of the tested plant such as fruits, seeds and bark will be conducted since these parts are used in

traditional medicine. Various studies revealed that bioactive compounds from plants exhibited selectivity to cancer cell lines, *C. papaya* could be subjected further studies utilizing other directly accessible cancer cell lines. Furthermore, it is recommended to use other solvent and method of extraction inorder to optimize the separation of the natural products. *Carica papaya* also warrant further investigation particularly on the isolation and characterization of potential novel bioactive compounds for drugs against cancer. The anti-cancer property of the ethanolic extract and ethyl acetate fraction of *C. papaya* leaves were determined through MTT assay. The ethanolic extract and ethyl acetate fraction of *C. papaya* revealed cytotoxic and anti-cancer activity against HCT- 116 human colon cancer cell line having IC_{50} values of 24.42 µg/ml and 34.87 µg/ml, respectively. On the otherhand, ethyl acetate fraction of the said plant manifested low cytotoxic activity againstmcF7 – Human breast cancer cell line and A549-Human adenocarcinoma cell line having IC_{50}>100 µg/ml of the two cell lines. Based on the results, the ethanolic extract and ethyl acetate fraction of *C. papaya* was established to significantly reduced HCT 116- Human colon cancer cell line proliferation, manifesting the cytotoxic/bioactive compounds in the said plant leaves. The low cytotoxic activity of the plant againstmcF7 and A549 cell lines could be attributed to the selective cytotoxicity of the cancer cells. The cytotoxic activity of *C. papaya* could be attributed to the presence of phytochemicals of the tested plant. Moreover, *C. papaya* deserves further investigation particularly on the isolation of potential novel biologically active compounds for drugs against cancer.

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