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Anticancer activity of ethnobotanicals of the Philippine Ilongot-Eǵongot community on human colon cancer cells (HCT-116)

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Abstract

Globally, colon cancer is one of the leading causes of cancer deaths. Colon carcinoma is one of the best-understood neoplasms from the genetic perspective, yet it remains the second most cause of cancer-related deaths. As one of the developing countries, Philippines possess a rich biodiversity that offers wide range of ethnobotanicals waiting to be utilized for cancer treatment. Ethanolic extracts of ten ethnobotanicals collected from the Ilongot-Eǵongot community of Bayanihan, Maria Aurora, and Aurora, Philippines were tested against human colon cancer cell line HCT-116. Cell viability and cytotoxicity was determined using the PrestoBlue® Assay. The assay revealed that *Premna odorata* (Local Name: Asedaong; IC50 0.8747 µg/ml), *Dillenia philippinensis*(Local Name: Katmon; IC50 0.6358 µg/ml), *Eleusine indica* (Local name: Pag; IC50 0.6653 µg/ml), *Mikania micrantha* (Local Name: Ola ola; IC50 0.8201 µg/ml), *Diplazium esculentum* (Local name: Pako Pako; IC50 0.7338 µg/ml), *Urena lobata*(Local name: Pukot; IC50 1.009 µg/ml), *Hydrocotyle vulgaris*(Local name: Gotukola; IC50 0.8788 µg/ml), *Ceiba pentandra* (Local name: Betek; IC50 1.04 µg/ml), *Adenanthera intermedia*(Local name: Kares; IC50 1.295 µg/ml) and *Stachytarpheta* sp. (Local Name: Luzviminda; IC50 1.322 µg/ml), showed anticancer activity against human colon cancer cell line (HCT-116). All extracts showed an IC50 <20 µg/mL which is considered as highly toxic.

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Introduction

Globally, one of the leading causes of cancer deaths is colon cancer. Colon carcinoma is one of the best-understood neoplasms from the genetic perspective, yet it remains the second most cause of cancer-related deaths (O'brien *et al*., 2007; Todaro *et al*., 2007).

The Philippines, with its rich biodiversity represents a huge potential of discovering drugs for the treatment of cancer. Approximately 16,223 plant species are reported with high plant endemism in which close to 6,300 are endemic. Among these are the ethnobotanicals. Ethnobotanicals are plants in the wild used by communities in the high lands for medicinal, religious, and other purposes.

In the recent years, there is an increasing trend in discovering drugs using ethnobotanicals (Balangcod & Balangcod, 2011). Given the diversity of indigenous tribes, ethnobotanical documents are relatively few, and only focused on well-known indigenous groups. Among the ethnic groups are the Ilongot-Ẹ́gongots, an indigenous tribe that resides in the high grounds of Nueva Ecija, Nueva Vizcaya, Aurora, and Quezon Province. Recent studies have shown the biological activities of Philippine ethnobotanicals (Alfonso, 2015; Jose, 2015; Divina, 2015; Judan Cruz *et al*, 2018; Limos *et al*., 2018; Barrogo *et al*, 2018; Padilla *et al*, 2018). Pooten *et al.* (2017) specifically reported anticancer activity of Philippine ethnobotanicals of the Igorot community from Imugan, Nueva Vizcaya which were tested against selected human cancer cell lines.

These ethnobotanicals exhibited high cytotoxic activities against the cancer cell lines used in the study. These studies offer insights on the biological activities of the ethnobotanicals with the prospects of drug discovery based on plants and plant-products. Hence, this study aimed to evaluate the anticancer activity of the ethnobotanicals of the Ilongot community of Bayanihan, Maria Aurora, and Aurora through determining the percentage cell viability per concentration of the plant extracts and its cytotoxic effect against human colon cancer cell line (HCT 116).

Materials and methods

Collection of plant samples

Plant samples of *Premna odorata* (Local Name: Asedaong), *Dillenia philippinensis*(Local Name: Katmon), *Eleusine indica* (Local name: Pag), *Mikania micrantha* (Local Name: Ola ola), *Diplazium esculentum* (Local name: Pako Pako), *Urena lobata* (Local name: Pukot), *Hydrocotyle vulgaris* (Local name: Gotukola), *Ceiba pentandra* (Local name: Betek), *Adenanthera intermedia* (Local name: Kares) and *Stachytarpheta* sp. (Local Name: Luzviminda), were collected in different areas of the Ilongot community in Maria Aurora, Aurora, Philippines. Plant parts relevant to its medicinal use were collected. Samples were properly labeled. Leaves, flowers, stems and fruits were plant pressed and was authenticated at Department of Biological Sciences, Central Luzon State University.

The location of the plants was determined using the Global Positioning System (GPS). Other environmental parameters were noted such as air temperature, soil pH, and humidity.

Ethanol extraction

The plant samples were washed three times; first, with tap water to remove the dirt on the surface of the plant material, then rinsed with distilled water and lastly with 70% (v/v) ethyl alcohol (Tan *et al*., 2013). After rinsing, the plant materials were air dried and powdered with the use of blender and extracted using 25 grams of each of the plant materials mixed with 250 mL of 80% ethanol for 72 hours. The mixture was filtered using Whatman paper no. 1 and the solvent was removed by rotary evaporation. It was stored in sterile ambers with stoppers under 0-5°C. Sterilization was done by centrifuge at 10,000 rpm for 30 minutes with syringe type filtration with pore size of 0.4µm.

Maintenance and preparation of cells

The bioactivity of the ethnobotanical extracts was tested on the human colon cancer cell line (HCT-116) which is routinely maintained at the Cell and Tissue Culture Laboratory, Molecular Science Unit, Center

for Natural Science and Ecological Research, De La Salle University.

Following the standard procedures of Freshney (2000), cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Gibco®, USA) containing 10% fetal bovine serum (FBS, Gibco®, USA) and 1x antibiotic-antimycotic (Gibco®, USA) and kept at 37°C with 5% CO_2 in a 98% humidified incubator. Upon reaching 80% confluence, the monolayer cultures was washed with phosphate-buffered saline (PBS, pH 7.4, Gibco®, USA), trypsinized with 0.05% Trypsin-EDTA (Gibco®, USA) and resuspended with complete fresh media. Cells were counted following standard trypan blue exclusion method using 0.4% Trypan Blue Solution (Gibco®, USA). Cells were later seeded in 100µL aliquots into 96-well microtiter plates (FalconTM, USA) using a final inoculation density of 1 x 104 cells/well. The plates were further incubated overnight at 37°C with 5% CO_2 in a 98% humidified incubator until complete cell attachment was achieved.

Cell viability assay

The bioassay method was done following the protocol of De Los Reyes (2015). The cytotoxicity of the ethnobotanicals were determined in a cell viability test using PrestoBlue® (Molecular Probes®, Invitrogen, USA). The bioassay was based on the presence of mitochondrial reductase in viable cells that converts the resazurin dye (blue and nonfluorescent) to resorufin (red and highly fluorescent). The conversion was proportional to the number of metabolically active cells and was determined quantitatively using absorbance measurements. To the monolayers in the microtiter plate, 100 µL of the extracts was added to corresponding wells at two-fold serial dilutions to make final screening concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 µg/mL. The treated cells were further incubated for 4 days at 37°C in 5% CO_2 and 98% humidity. Twenty microliters of PrestoBlue® was added to each well. The cells were be incubated for 1 hr at 37°C in 5% CO_2 and 98% humidity. Wells with no sample added served as negative controls while wells with ZeocinTM (Gibco®, USA) served as positive control. Absorbance measurements were carried out using BioTek ELx800 Absorbance Microplate Reader (BioTek® Instruments, Inc.) at 570 nm and normalized to 600 nm values (reference wavelength).

Absorbance readings were used to calculate for the cell viability for each sample concentration using the equation:

$$Cellviability\% = \frac{(Absorbance of Treated Sample - Absorbance of Blank)}{(Absorbance of Negative Control - Absorbance of Blank)} x100$$

Inhibitory concentration was used to compare the threshold values. Resulting IC_{50} values having ≤30 µg/mL were considered as having significant cytotoxic effects.

Statistical analysis

The cell viability assay data was analyzed by nonlinear regression using GraphPad Prism 6.05 (GraphPad Software, Inc.) to estimate the half maximal inhibitory concentration IC_{50}.

Results and discussion

Cell viability and cytotoxicity assay

Fig. 1 shows that the cell viability under different plant extracts have been reduced by 80% and followed a downward slope. All of the plant extracts have a significant decrease pattern of viability as the concentration increases. Cell viability shows the percentage of living cells after exposure to different plant extracts. Two extracts namely *P. odorata* and *D. philippinensis*showed least cell viability having (17.765%) followed by *D. esculentum* (19.722%), *E.indica*(20.362%), *M. micrantha* (21.567%), *Stachytarpheta*sp. (23.035%), *U. lobata* (23.863%), *C. pentandra* (24.277%), *H. vulgaris* (24.427%), *A. intermedia*(27.401%). As expected, the cytotoxicity index shows an upward slope, the higher the concentration of treatment the higher the plant cytotoxicity (Fig. 1). All the plant extracts tested showed cytotoxicity towards HCT-116. Two plant extracts had the highest cytotoxicity of 82.235% namely *P. odorata* and *D. philippinensis*.

Table 1. Inhibitory concentration of ethnomedicinal plant extracts against human colon cancer cell line(HCT-116).

Treatments	IC_{50} 30µg/ml	Toxicity
Zeocin (Control)	1.755 µg/ml	Highly toxic
Stachytarpheta sp.	1.322 µg/ml	Highly toxic
Adenanthera intermedia	1.295 µg/ml	Highly toxic
Ceiba pentandra	1.04 µg/ml	Highly toxic
Hydrocotyle vulgaris	0.8788 µg/ml	Highly toxic
Urena lobata	1.009 µg/ml	Highly toxic
Diplazium esculentum	0.7338 µg/ml	Highly toxic
Eleusine indica	0.6653 µg/ml	Highly toxic
Mikania micrantha	0.8201 µg/ml	Highly toxic
Dillenia philippinensis	0.6358 µg/ml	Highly toxic
Premna odorata	0.8747 µg/ml	Highly toxic

Inhibitory concentration of plant extracts

Table 1 shows the inhibitory concentration of the ethanolic extracts against colon cancer cell line (HCT 116). American National Cancer Institute established that the values of IC_{50} should be less than 30 µg/ml to be considered toxic. The ten plants used in the study are considered highly toxic with values <2 µg/ml with all the plants having values of higher toxicity compared to the positive control, Zeocin, towards the human colon cancer cell line (HCT 116).

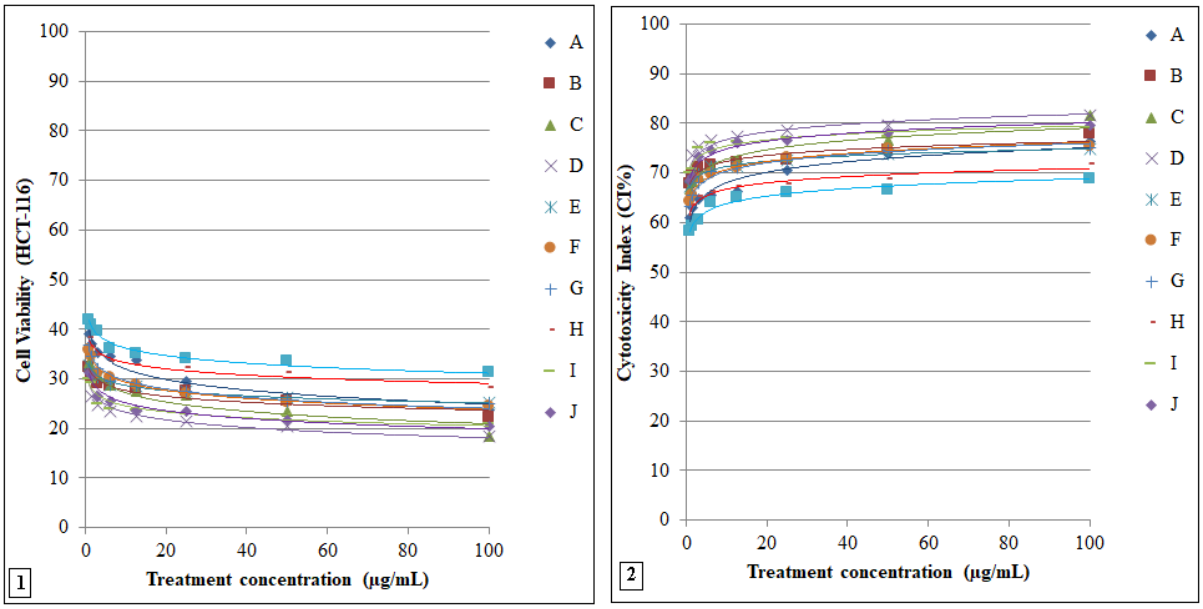

Fig. 1. Inhibitory (1) and Cytotoxic Activity (2) of (A)*Stachytarpheta*sp.(B)*M. micrantha* (C) *P. odorata*(D) *D. philippinensis*(E) *H. vulgaris*(F) *U. lobata*(G)*C. pentandra*(H) *A. intermedia*(I) *E. indica*(J) *D. esculentum* and Zeocin on human colon cancer cell line (HCT-116).

These plants showed low level of cell viability and a high level of cytotoxicity index after treatment of the plant ethanolic extracts against colon cancer cell line (HCT 116). High cytotoxicity may be attributed to the plants phytochemical composition. Phytochemicals that are considered as anticancer compounds includes flavonoids,alkaloids, tannins, saponins, and phenols. Eight of the plants used were found to haveflavonoids (*Stachytarpheta* sp., *M. micrantha*, *P. odorata*, *H. vulgaris*, *U. lobata*, *C. pentandra*, *E. indica*, *D.*

esculentum) present in the different parts of the plants as well as saponins (*D. philippinensis*, *P. odorata*, *U. lobata*, *D. esculentum*, *Stachytarpheta* sp., *H. vulgaris*).

Only four plants (*C. pentandra, E. indica*, *U. lobata*, *Stachytarpheta* sp.) used in the study contain alkaloids that is potentially lethal if accumulated in large amounts. Phenols are strong antioxidants and known to inhibit cancer related pathways leading to induce caspase-mediated apoptosis activity (Spilioti *et al*., 2014). Alkaloids are anticancer agents which inhibit the enzyme topoisomerase which is involved in DNA replication, inducing apoptosis and expression of the p53 gene Mohan *et al.* (2012) and are present in *C. pentandra*, *E. indica*, *U. lobata* and *Stachytarpheta* sp. this compound is biologically active and is lethal at large amounts and may cause serious addictions (Mohan *et al*., 2012).

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