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ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF SELECTED PLANT EXTRACTS AGAINST HUMAN NON-SMALL CELL LUNG ADENOCARCINOMA (A549), HUMAN COLON CARCINOMA CELLS (HCT116) AND CHINESE HAMSTER NORMAL OVARY CELLS (AA8)

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ABSTRACT: Chemotherapy and radiation are the most prescribed methods of cancer treatments but these are highly toxic and also are carcinogenic thus alternative cures are being explored especially on the use of cytotoxic substances isolated from natural sources like plants known for their ethnomedicinal properties. Natural chemopreventive agents such as antioxidants isolated from plants are argued to be important in suppressing or reversing carcinogenesis and to prevent the development of invasive cancers thus research studies are now currently geared in the evaluation and determination of the cytotoxicity and antioxidant activity of plant extracts to serve as ideal cure. Since the acquisition and processing of highly cytotoxic and selective effects of plant-based compounds to cancer cells are still limited, this study was conducted on eight plant species known to have folkloric medicinal properties. The leaf ethanolic extracts were examined for their antioxidant properties and cytotoxicity against Human Non-Small Cell Lung adenocarcinoma (A549), Human Colon Carcinoma Cells (HCT116) and Chinese Hamster Normal Ovary Cells (AA8). Antioxidant properties were observed to be high in seven species. Only one species *P. edule* to have low antioxidant property but high toxicity to HCT116 cancer cell lines. Three other species *C. ovatum*, *F. nota* and *P. odorata* were both having high antioxidant properties and cytotoxicity to cancer cell lines HCT116 while four species *S. contorta*, *T. copelandii*, *C. ramiflora* and *P. arborea* have high antioxidant but relative very low toxicity to HCT116 cancer cell lines. While two species namely, *C. ovatum* and *P. edule* were cytotoxic to HCT116 cancer cell lines, only *C. ovatum* was found to be cytotoxic for both A549 cancer cell lines thus could be a good candidate as source of chemotherapeutic and chemopreventive compounds. The results that showed seven out of the eight species have high percentage of antioxidants can be argued that these species are candidate species as sources of chemopreventive compounds. Further studies are still needed to determine the compounds from these species that confer selective cytotoxicity to cancer cell lines.

INTRODUCTION: Cancer is the prevailing cause of death in industrialized countries and second in developing countries [1]. These include lung cancer in males and breast cancer in females followed by without any specific order, by colorectal and lung or prostate cancers in males and colorectal and lung cancers in females in industrialized countries, and by stomach and liver cancers in males and cervix and lung cancers in females in developing countries [1].

A survival analysis of cancer patients from 1990 to 2003 in Africa, Asia and Central America, indicated significantly lower survival rates in Gambia, Uganda, the Philippines and India than for those diagnosed in South Korea, Hong Kong, Singapore and Turkey [1, 2].

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Chemotherapy and radiation are the most prescribed methods of cancer treatments but these are highly toxic and also are carcinogenic thus alternative cures are being explored especially on the use of cytotoxic substances isolated from natural sources like plants known for their ethnomedicinal properties. In the Philippines, medicinal plants are considered to be one of its natural living treasures since their use for primary health care is a substantial help in meeting the drug requirements of the country. However, their medicinal use has been based mostly on ethnomedicinal grounds thus there is a need for scientific validation of such ethnomedicinal knowledge.

Research studies are currently geared in the evaluation and determination of the cytotoxicity and antioxidant activity of plant extracts. These are important in obtaining possible plant-based anti-oxidant-supplements which are not only vital in fighting oxidative damage but also as source of chemo preventive drugs. A good number of these studies have been dedicated to isolation and discovery of such compounds to serve as the ideal cure [3, 4, 5, 6, 7, 8, 9]. Easy acquisition and processing and highly cytotoxic and selective effects to cancer cells however remains elusive [10] thus, this study is an attempt in the continuing search for these kinds of plant resources.

Eight Philippine plants namely, Balitbitan (*Cynometra ramiflora*), Bolon (*Platymitra arborea*), Lanipau (*Terminalia copelandii*), Pangi (*Pangium edule*), Tibig (*Ficus nota*), Alagau (*Premna odorata*), White Lauan (*Shorea contorta*) and Pili (*Canarium ovatum*) were selected as these plants were reported to have ethnomedicinal properties. *C. ramiflora* is reported to have ethnomedicinal properties [11, 12, 13, 14, 15]. Its roots are considered purgative, seeds and leaves anti-herpetic, leaves used to make a lotion for skin diseases, oil drawn from the seeds are also used for skin affections. Another plant *P. arborea* has edible ripe fruit and its folkloric uses include boiled fruit used for fevers, decoctions of fruit used are for amenorrhea and antidysentery while that of the bark with dried leaves of garlic used are for urticaria and diabetes. It also has been reported to have antimicrobial properties [16, 17]. *P. edule* is a wild plant used as folk medicine for wound healing. It's cold water infusion is used in Indonesia to counteract putrefaction and expel parasites. Its seed extracts were also found to possess antioxidative activity and some also have antimicrobial properties [18, 19, 20] *Ficus nota* and many other *Ficus species* were reported to be used for tumor and cancer treatments [21, 22, 23, 24] and in the treatments of warts [25, 26, 27, 28, 29]. Only the species *T. copelandii* investigated in this study is popular as timber and only reported through the word of mouth that are also used for treating basic ailments by the local people. *P. odorata* is reported to have sudorific, pectoral, carminative, anti-microbial, cardiotonic, anticoagulant, hepato-protective, antitubercular, antitumor properties [30-39].

S. contorta is reported to have its decoction of the bark used for cough, as antipyretic, tonic and astringent and a decoction of the wood inhibits tumor [40]. The *S. contorta* plant is used as a decoction of the bark to be used for the treatment of cough, as an antipyretic when combined with the leaves and inhibits tumor from a decoction of the wood. For *C. ovatum* however, seed kernel is eaten for the treatment of laxative effect, It is eaten as tolerated. Emulsion from crushed kernels has been used as substitute for infant's milk. It is also used for making medicinal ointments [40]. It was also reported to have antimutagenic and anti-melanogenesis inhibitory activity [41, 42]. The extracts from the bark is also used in the treatment of malaria, the leaves are used in the treatment of vertigo and raw nuts are a purgative [43].

As to the possible properties of these eight plants to possess antioxidant, cytotoxic and anticancer properties, we use DPPH and MTT assays. We specifically investigate whether the plant extracts have effects against colon and lung cancer cell lines and if they induce death on Chinese hamster non-cancer ovary cells (AA8). It is argued that the outcomes of the study would be a driving force for the development of ethnomedicines with strong antioxidant properties and as indicators in the evaluation of a future chemopreventive drug.

MATERIALS AND METHODS: This study focuses on the evaluation of the leaves extracts of the eight plant species *C. ramiflora, P. arborea, T. copelandii, P. edule, F. nota, P. odorata, S. contorta* and *C. ovatum* using DPPH and MTT assays. Furthermore, this study attempts to determine which among the leaves extracts has the

highest percentage antioxidant activity and which of them show cytotoxic activity against HCT116 cell line or colon cancer and A549 cell line or lung cancer and to AA8 non-cancer ovary cells. The laboratory analysis was done at the Institute of Biology, College of Science in the University of the Philippines, Diliman, Quezon City.

Chemicals and Reagents: All chemicals and reagents used were of analytical grade. 1,1-Diphenyl- 2- picrylhydrazyl (DPPH) radical available from Institute of Biology, College of Science- University of the Philippines Diliman, Quezon City, Philippines.

Preparation of Ethanolic Crude Leaf Extracts: The leaves of *C. ramiflora*, *P. arborea*, *T. copelandii*, *P. edule*, *F. nota*, *P. odorata*, *S. contorta* and *C. ovatum* from Aras-asan Timber Company in Aras-asan, Cagwait, Surigao del Sur in the Philippines and were identified by Mr. Ramon Bandong of the University of the Philippines - Diliman Herbarium. These leaves were air dried until crisp dry and homogenized using an Osterizer blender. The homogenized leaves were soaked in 95% ethanol for three days. After soaking, the leaf suspension was filtered using Whatmann filter paper. The filtrate was run in a rotary evaporator at 40 °C to separate the solvent and obtain the crude extract. Four milligrams of each of the crude leaf extracts were obtained and dissolved in one milliliter of dimethyl sulfoxide (DMSO). These solutions were placed in microfuge tubes and stored in the refrigerator until use in subsequent assays.

Culture, Maintenance and Washing of Cells: The Human Non-Small Cell Lung adenocarcinoma (A549), Human Colon Carcinoma cells (HCT116) and Chinese hamster Normal Ovary cells (AA8) were obtained from American Type Culture Collection (ATTC, Manassas, Virginia, USA). A549 cells were maintained in a culture medium composed of 88% F-12 K medium with L-glutamine, 10% Fetal bovine serum (FBS), 1% antibiotic-antimycotic, and 1% sodium bicarbonate. HCT116 cells were maintained in a culture medium composed of 87.5% modified McCoy's 5A medium with L-glutamine, 10% Fetal bovine serum (FBS), 1% antibiotic-antimycotic, and 1.5% sodium bicarbonate. AA8 cells were maintained in a culture medium composed of 87% RPMI medium with two grams sodium bicarbonate per liter, 10% Fetal bovine serum (FBS) and 1% antibiotic-antimycotic. All cells were kept in an incubator set at 37 °C and 5% CO_2.

The cell cultures were washed from time to time to remove non-adherent dead cells and metabolites that have accumulated in the flask as seen via microscopic examination. In washing cells, the old medium was first removed then the culture was washed with 8 ml sterile 1X phosphate-buffered saline (PBS). Five ml of fresh medium was then added to the flask. All the steps in the process were performed under the laminar flow hood.

A549 cells, HCT116 cells and AA8 cells were subcultured at 85% - 95% confluence. This was done inside the laminar flow hood. To subculture, old medium was pipetted out of the flask and the cells were washed with 8 ml sterile 1X PBS. To dislodge the cells, 750 µl of 1X trypsin was added to A549 and HCT116 cells, while the same amount of 0.5X trypsin was added to AA8 cells. The flasks were smacked hard to facilitate detachment of cells. When the cells were finally observed to have sufficiently detached, 3 ml of the culture medium was added to neutralize trypsin. Cell suspension at 2×10^4/ml was retained in the flask; the rest was discarded. Five milliliters of fresh medium was then added to the subculture.

MTT Cytotoxicity Assay: The MTT cytotoxicity assay performed in this study was adapted from Mosmann [44]. In detail, AA8, A549 and HCT116 cells were seeded separately at 3.5×10^4 cells/ml in sterile 96-well microtliter plates. The plates were incubated overnight at 37 °C and 5% CO_2.

The 4 mg/ml extracts were serially diluted to concentrations 1000 µg/ml, 500 µg/ml, 250 µg/ml and 125 µg/ml in a master dilution plate (MDP). From the MDP, 10 µl were obtained and dispensed onto the plated cells to obtain the final screening concentrations 50 µg/ml, 25 µg/ml, 12.5 µg/ml and 6.25 µg/ml.

Cells treated with Doxorubicin served as positive control while those treated with DMSO, the solvent of the extracts, served as negative control. Three replicate wells were used per concentration. The treated cells were then incubated for 72 h at 37 °C and 5% CO_2.

After incubation the media was removed and 20 µl 3-(4, 5-dimethylethylthiazol -2 -yl) -2, 5-diphenyl tetrazolium bromide (MTT) at 5 mg/ml PBS was added. The cells were again incubated at 37 °C and 5% CO_2 for four hours, after which 150 µl DMSO was added to each well. Absorbance was read at 570 nm. The concentration required in killing 50% of the cell population or the inhibition concentration 50 (IC_{50}) was computed using linear regression of the graph of absorbance against concentration. Three trials with three replicates per concentration were done for extracts that showed cytotoxic activity while non-cytotoxic extracts, were subjected to at least one trial.

DPPH Radical Scavenging Activity: The DPPH Free Radical Scavenging Assay performed in this study was adapted from Molyneux [45]. A 300 µM free-radical solution was prepared by dissolving 1.2 mg of 2, 2-diphenyl -1-(2, 4, 6-trinitrophenyl) hydrazyl (DPPH) in 10 ml absolute ethanol. The solution at 95 µl was dispensed to 96-well microliter plates. Gallic acid was used as positive control while DMSO, the solvent of the extracts, was used as negative control. Five microliters of the controls and extracts were added to the wells to make a final volume of 100 µl. There were three replicates for each control and extract.

The plate was then covered with a plate sealer, mixed gently and wrapped with aluminum foil. It was incubated at 37 °C and 5% CO_2 for 45 min. After incubation, absorbance was read at 520 nm. Based on the absorbance readings, free radical inhibition of the leaf extracts was computed using the formula:

$$\% \text{ Inhibition } \frac{\text{Absorbance}_{\text{DMSO}} - \text{Absorbance}_{\text{extract}}}{\text{Absorbance}_{\text{DMSO}} - \text{Absorbance}_{\text{gallic acid}}} \times 100$$

The flow of the research flow is summarized in the **Fig.** below:

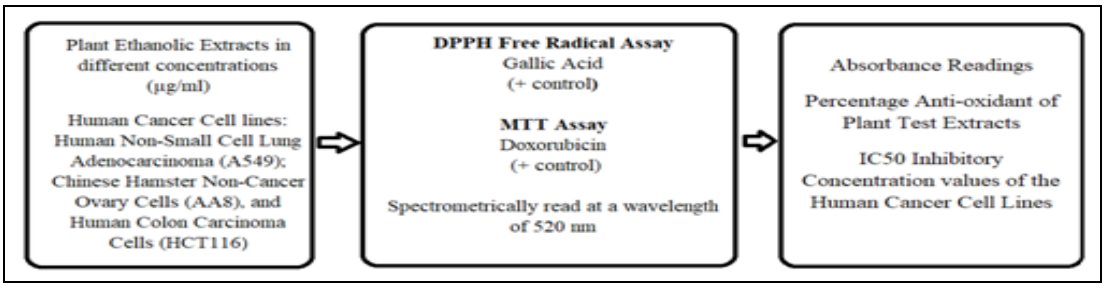

FIG. 1: THE FLOW CHART OF THE RESEARCH

Statistical Analysis: All the assays were carried out in triplicate. Experimental results were expressed as means ± standard deviation (SD) of three parallel measurements. Normality of distribution and homogeneity of the variances of means was assessed through the Levene's test. Means was then analyzed with One-way Analysis of Variance (ANOVA). The results of ANOVA from data sets with unequal variances of means were confirmed using the Brown-Forsythe test. Post hoc tests (Games-Howell test for data sets with unequal variance and Tukey's Honestly Significant test for data sets with equal variance) were then be performed to determine significant differences between means. P-values less than 0.05 were taken as indicators of significant differences between compared means. Three independent trials with at least two replicates each were performed for each experiment to maintain statistical significance. All statistical analysis was performed using the SPSS Statistics 17.0 software.

Data Interpretation: Absorbance values that are lower than the control cells indicate a reduction in the rate of cell proliferation. Conversely, a higher absorbance rate indicates an increase in cell proliferation. The IC_{50} is the inhibitory concentration at which 50% of the cells die. If the IC_{50} is 20, it means that the plant extract with a concentration of 20 micrograms per ml solvent can kill 50% of the cells in the population. The IC_{50} values less than 10 are the ones considered as cytotoxic and below 20 are considered as having the potential to be cytotoxic.

RESULTS: The antioxidant activity of the extracts determined using a DPPH scavenging assay is presented in **Table 1**. The DPPH assay evaluates the ability of antioxidants to scavenge free radicals which are known to be a major factor in biological damages caused by oxidative stress and provide a reliable information concerning the antioxidant ability of the tested compounds and in our study

determined the ability of ethanolic extracts of *C. ramiflora*, *P. arborea*, *T. copelandii*, *P. edule*, *F. nota*, *P. odorata*, *S. contorta* and *C. ovatum* to scavenge DPPH.

TABLE 1: STATISTICAL EVALUATION OF THE HOMOGENEITY OF ANTIOXIDANT PROPERTIES OF THE EIGHT PLANT EXTRACT

ANOVA (Antioxidant)					
Test of homogeneity of variances (Antioxidant)					
Levene Statistic	df1		df2		Sig.
6.964	8		18		.000
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12547.159	8	1568.395	2.454	.000
Within Groups	869.892	18	48.327		
Total	13417.051	26			

TABLE 2: ANTIOXIDANT SCAVENGING ACTIVITY FOR EACH EXTRACT. [THE PERCENTAGE INHIBITION OF DPPH RADICAL WAS CALCULATED BY COMPARING THE RESULTS OF THE TEST EXTRACTS WITH THOSE OF THE CONTROL (GALLIC ACID)]

Extracts	% Antioxidant activity
Gallic Acid	100.00 ± 0.11c
Canarium ovatum	91.99 ± 1.87bc
Pangium edule	26.84 ± 13.82*a
Ficus nota	79.01 ± 9.64*b
Premna odorata	97.36 ± 1.38bc
Shorea contorta	98.18 ± 3.33bc
Terminalia copelandii	97.42 ± 0.44bc
Cynometra ramiflora	83.30 ± 0.13bc
Platymitra arborea	80.88 ± 0.45bc

*. The mean difference is significant at 0.05 levels.

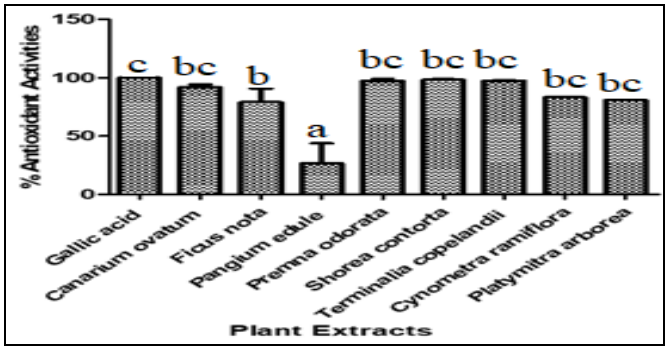

FIG. 2: MEAN (± SD) INHIBITION OF THE FREE RADICAL DPPH BY THE ETHANOLIC CRUDE LEAF EXTRACTS. [THE PERCENT INHIBITION OF THE PLANT EXTRACTS WERE SET TO 0 AS THE SAID EXTRACTS PRODUCED NEGATIVE VALUES IN THE DPPH ASSAY, INDICATING THE ABSENCE OF ANTIOXIDANT ACTIVITY. SAMPLES WITH THE SAME LETTER OCCUPY THE SAME GROUP AS DETERMINED BY DMRT

Results obtained showed significant variations in antioxidant activities among the plant extracts. A closer look at the results show that *S. contorta* exhibited the highest percentage antioxidant activity of 98.18 ± 3.33 compared to gallic acid and was followed by *T. copelandii* (97.42 ± 0.44) and *P. odorata* (97.36 ± 1.38). The other plant extracts indicate good sources of natural antioxidant while there are significantly lower percentage antioxidant activities of both *P. edule (*26.84 ± 13.82) and *Ficus nota* (79.01 ± 9.64) **Table 2**, **Fig. 2**.

Since a good free radical scavenger must register an inhibition of >80%; 50-80% inhibition is considered moderate and <50% is a very weak radical scavenger 44 (Mosmann, 1983), the DPPH results shown in **Table 1** indicate that extracts from *C. ovatum*, *P. odorata*, *S. contorta*, *T. copelandii*, *C. ramiflora* and *P. arborea* as good sources of free radical scavengers and therefore of natural antioxidants. *F. nota* extracts had moderately strong free radical scavengers and antioxidant activities while *P. edule* extracts had the weakest.

The findings of the present study clearly demonstrated the ability of *C. ramiflora*, *P. arborea*, *T. copelandi*, *P. odorata*, *S. contorta* and *C. ovatum* and even *F. nota* to effectively scavenge free radicals. For the cytotoxicity tests, the IC_{50} is the inhibitory concentration of the plant extracts at which 50% of the cells die. The negative control was the DMSO and it was not toxic to the cells and the values of absorbance were only used to compute for the IC_{50}. The IC_{50} values less than 10 are considered highly cytotoxic.

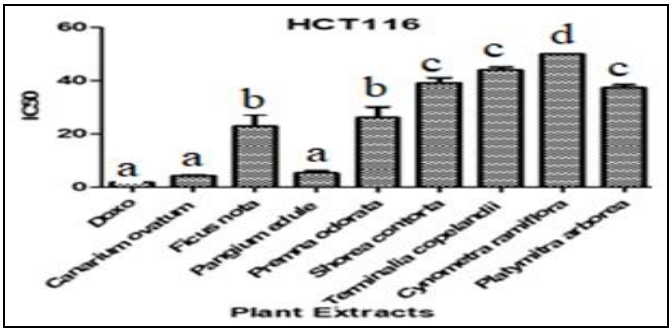

FIG. 3: MEAN (± SD) IC_{50} OF THE ETHANOLIC PLANT EXTRACTS AGAINST HCT116 CELLS. [SAMPLES WITH THE SAME NUMBER OCCUPY THE SAME GROUP AS DETERMINED BY DMRT]

The IC_{50} values below 20 are considered as having the potential to be cytotoxic. **Table 2** shows the statistical evaluation of the homogeneity of cytotoxic properties of the eight plant extracts against the Human Colon Carcinoma Cells HCT116 cells. HCT116 is a very sensitive cell line. It can be seen that there were variations in the cytotoxic effects of the plant extracts **Table 3**, **Fig. 3**. *C. ovatum* and *P. edule* crude ethanolic extracts displayed the most cytotoxic average as compared to the control Doxo. Higher inhibitory activities were observed in *S. contorta*, *T. copelandii* and *P. arborea*. These plant extracts were potentially cytotoxic to colon cancer cells compared to cells treated with the positive control, doxorubicin. *C. ramiflora* (50.00 µg/ml) showed no linear interpolation and was not cytotoxic.

TABLE 3: STATISTICAL EVALUATION OF THE HOMOGENEITY OF CYTOTOXIC PROPERTIES OF THE EIGHT PLANT EXTRACTS AGAINST HCT116 CELLS

Test of Homogeneity of Variances					
HCT116					
Levene Statistic		df1	df2		Sig.
5.512		8	18		.001
ANOVA					
HCT116					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8050.360	8	1006.295	69.998	0.000
Within Groups	258.769	18	14.376		
Total	8309.129	26			

TABLE 4: IC_{50} VALUES (mg/ml) OF THE PLANT EXTRACTS AGAINST SELECTED CANCER CELL LINES HCT116 AND A549 AND THE NON-CANCER CELL LINE AA8 AS ASSESSED BY THE MTT ASSAY. [NO L.I. (NO LINEAR INTERPOLATION) INDICATES NO INHIBITORY ACTIVITY AGAINST A PARTICULAR CELL LINE FOR AT LEAST TWO TRIALS. DATA ARE PRESENTED HERE AS MEANS + S.D]

Extracts	IC_{50} (µg/ml)		
	HCT116	**A549**	**AA8**
Doxorubicin	1.88 ± 0.11	2.37 ±0.10	2.05 ± 0.06
C. ovatum	4.39 ± 0.59	5.36 ± 0.26	4.14 ± 0.35
P. edule	5.40 ± 1.56	36.18 ± 1.81*	29.26 ± 0.91*
F. nota	22.95 ± 7.75		
P. odorata	26.18 ± 6.95		
S. contorta	39.15 ±3.33*		
T. copelandii	44.00 ±1.90*		
C. ramiflora	50.00 ± 0.00 NLI*		
P. arborea	37.34 ± 1.87*		

*. The mean difference is significant at 0.05 levels

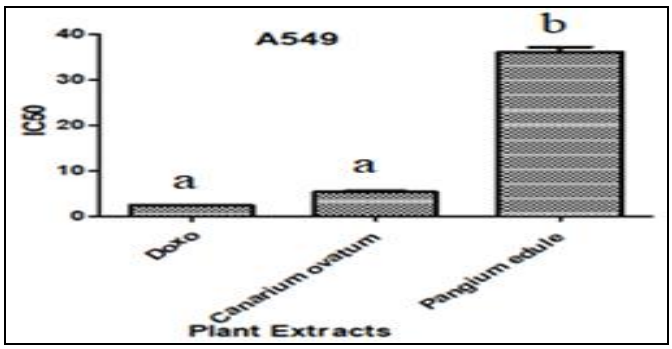

FIG. 4: MEAN (± SD) IC_{50} OF THE ETHANOLIC PLANT EXTRACTS AGAINST A549 CELLS. SAMPLES WITH THE SAME NUMBER OCCUPY THE SAME GROUP AS DETERMINED BY DMRT

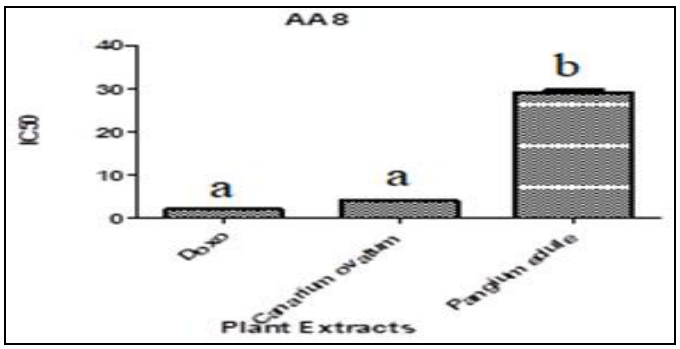

FIG. 5: MEAN (± SD) IC_{50} OF THE ETHANOLIC PLANT EXTRACTS AGAINST AA8 CELLS. SAMPLES WITH THE SAME NUMBER OCCUPY THE SAME GROUP AS DETERMINED BY DMRT

With the exception of two species *C. ovatum* and *P. edule*, other plant extracts that showed poor cytotoxic activities to HCT 116 cancer cells were not evaluated further for their cytotoxicity to the human non-small cell lung adenocarcinoma (A549) and the Hamster Non-Cancer Ovary Cells (AA8) cell lines. *C. ovatum* and *P. edule* which displayed the most cytotoxicity against HCT116 cell lines were further tested against the Human Non-Small Cell Lung adenocarcinoma A549 cell lines. The

evaluation of *C. ovatum* and *P. edule* cytotoxicity to A549 and AA8 cell lines showed that AA8 cells incubated with *C. ovatum* extract demonstrated significantly lower IC_{50} value compared to AA8 cells treated with *P. edule* ethanolic extracts **Table 4**, **Fig. 4** and **5**. This demonstrates that *P. edule* had a minimal harm on AA8 normal cells than *C. ovatum* which was highly cytotoxic.

DISCUSSION: Chemopreventive agents are argued to be important in suppressing or reversing carcinogenesis and to prevent the development of invasive cancers [46]. Plants are good sources of these agents in the form of antioxidants which inhibits the oxidation of other substances [47]. Oxidation reactions may produce free radicals-species with one or more unpaired electrons- which are involved in the initiation and promotion of carcinogenesis [48]. Of the eight plant samples examined, antioxidant property of the seven species ranged from 79 to 98% which are relatively high **Table 1**. Only 1 plant species *P. edule* showed its extracts to have a very low antioxidant property. *P. edule* ethanolic extract showed the least antioxidant property with only 27%. This may be due to the use of the plant leaves as source of the extract. Other studies used seed extracts of the plant and reported these to possess antioxidative activity [20, 49]. What is interesting to note from this study that the leaf extract was highly cytotoxic to the HCT116 cancer cell lines. It can be argued from this result that the cytoxicity of the leaf extract from the plant contain hydrogen cyanide that is deadly poisonous just like just like those found in the fresh fruit and seeds [50, 51, 52].

P. edule crude leaf extract is cytotoxic against HCT116 cells, and is highly selective towards this type of cancer cells over non-cancer cells. The result of *P. edule* confirms the result which has been analyzed to be cytotoxic against colon cancer cell line HCT-8, melanoma cell line MDA/MB-435, and glioblastoma cell line SF-295 [9]. Our results showing cytotoxicity against non-cancer cells (AA8) are nonpolar and soluble in hexane thus removing these compounds would likely improve the extract's selectivity towards the cancer cells.

Ethanolic extract from *C. ramiflora* is high in antioxidants based from the results of this study. It was however shown to have the least cytotoxic activity against HCT116 when compared with the other seven species. This plant was reported to be used against human gastric, colon and breast cancer but exerted low toxicity against mouse fibroblast indicating selective cytotoxicity against different cancer cell lines [11]. This study including that of the current results of our study may be attributed to the presence of high antioxidant activity but least cytotoxicity to the HCT116 cancer lines of the ethanolic leaf extract.

For *P. odorata*, results in this study show the ethanolic leaf extract has high antioxidant and cytotoxicity to HCT116 cancer cell lines. Our study may confirm some studies which reported chemopreventive activities of two amorphous powders identified as flavone glycones from the crude ethanolic extract of the leaves of the plant [36].

Studies have shown *P. odorata* also reduce *in-vitro* cell viability of a human leukemia cell line by more than 50% when exposed to broad spectrum light [32]. Likewise, a study on the alcohol extract of the plant showed significant antitumor activity [33] while another study showed the leaves and bark hexane fractions to be highly cytotoxic against the HCT116, MCF-7 and A549 cancer cell lines although it was shown the bark hexane extract has highest selectivity index for the three cancer lines [39]. This current study show *C. ovatum* ethanolic extracts of the leaves was having high antioxidant properties and high toxicity to HCT116, A549 and AA8 cell lines. These ethanolic extracts can be argued to be a potential source of chemotherapeutic and chemopreventive compounds.

In a study conducted on micronucleated poly-chromatic erythrocytes, the methanolic extract of *C. ovatum* showed the most promising activity by reducing their numbers and this study also established its antimutagenic activity and may be valuable in cancer chemoprevention [53]. On the basis of the results obtained in the DPPH assay, it confirms with the which previously reported *C. ovatum* to possess potent antioxidant activity based on the DPPH assay [54, 55].

For *Ficus species*, our study on *F. nota* shows the ethanolic leaf extracts both has antioxidant and cytotoxic activity to HCT116 cancer cell lines. These results may confirm those claims on the medicinal properties of the plant where it was

reported to be used as cure for cancers [21, 23, 24]. Many studies have shown the isolation of anti-neoplastic, anti-inflammatory and antioxidant compounds in the plant [56-63]. A study conducted on the chemical composition of unripe fruits has shown the presence of compounds that are known to exhibit antioxidant, prooxidant, anti-inflammatory, antibacterial, hypocholesterolemic, anti-diabetic, cytotoxic, and immunomodulatory effects [64, 65, 66, 67, 68]. This antioxidant and pro-oxidant nature of *F. nota* is supported by several studies on related species *F. fiskei* and *F. ulmifolia* and *F. odorata* [69].

For *P. arborea* and *S. contorta*, studies on their medicinal properties were limited to their folkloric use in the treatment of fevers, amenorrhea, urticaria, dysenteric and diabetes. *P. arborea* was shown having exceptionally high values of antimicrobial activity [16] while the other species *S. contorta*, folkloric studies revealed the decoction of the bark cures cough, with leaves used as antipyretic, tonic and astringent while a decoction of the wood is used to inhibit tumor. The current study has shown that the ethanolic extracts of these two species although not cytotoxic against either HCT116 or A549 cell lines, had high free radical scavenging activity. This implies the potential of the extracts to be used as chemopreventive agents against the onset of cancer.

CONCLUSION: This study determining the antioxidant and cytotoxicity of the ethanolic leaf extracts of eight plant species show variations among species in their properties. One species *P. edule* to have low antioxidant property but high toxicity to HCT116 cancer cell lines. Three other species *C. ovatum, F. nota and P. odorata* were both having high antioxidant properties and cytotoxicity to cancer cell lines HCT116 while four species *S. contorta*, *T. copelandii*, *C. ramiflora* and *P. arborea* have high antioxidant but relative very low toxicity to HCT116 cancer cell lines.

While two species namely, *C. ovatum* and *P. edule* were cytotoxic to HCT116 cancer cell lines, only *C. ovatum* was found to be cytotoxic for both A549 cancer cell lines. Further studies however are still needed to determine the compounds that confer selective cytotoxicity to cancer cell lines. The results nevertheless show that the high percentage of antioxidants in seven out of the eight species can be argued as potential sources of chemopreventive compounds.

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