

Evaluation of Cytotoxicity and Genotoxicity of *Canarium ovatum* Engl. (Burseraceae) and *Cocos nucifera* L. (Arecaceae) Ethanolic Bark Extracts

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Abstract

To further elucidate the potential of the pili and coconut barks in terms of their anticancer activity, their crude extracts were subjected to the sea urchin fertilization assay and *Allium cepa* test. These tests were conducted in order to determine the cytotoxicity and genotoxicity activities against the test subjects. The generated IC₅₀ values are 0.54 mg/ml and 0.20 mg/ml for *Canarium ovatum* and *Cocos nucifera*, respectively. The sea urchin egg cells possess strong sensitivity against toxic agents as well as onion root cells which display the genetic correspondence of the cells affected by noxious substances. *Canarium ovatum* crude bark extracts exhibited a significant inhibition activity ($p < 0.0001$) against the sea urchin egg cell fertilization whereas the *Cocos nucifera* bark extracts did not show any significant inhibition. In terms of its genotoxicity, *Canarium ovatum* crude bark extracts exhibited a significant inhibition on the mitotic indexes and increased the frequency of aberrations in the onion root cells whereas no significant inhibition on the mitotic indexes of the root cells was observed among those treated with *Cocos nucifera*. Phytochemical screening results of *Canarium ovatum* crude bark extracts presented abundant compounds such as flavonoids, phenols, tannins and with traces of saponins. For the *Cocos nucifera* crude bark extracts, compounds such as saponins were found abundant with traces of flavonoids, phenols, tannins, diterpenes and terpenoids. The results indicated that *Canarium ovatum* possess anti-proliferative potential as exhibited in the two assays while *Cocos nucifera* possess toxic potentials at certain concentrations. Further studies are needed to validate the results of the present investigation.

Keywords: *Pili*, *Coconut*, *Cytotoxicity*, *Genotoxicity*, *Anticancer*, *Sea urchin*, *Allium cepa*

Introduction

According to the World Health Organization in 2017, cancer is the second leading cause of death and responsible for 8.8 million deaths in 2015. The global statistics demonstrates that 1 in 6 deaths is affected by cancer and approximately 70% of mortality caused by cancer transpire in low- and middle-income countries. In addition, the World Health Organization also stated that in the year 2015, approximately 65% to 80% of the population of developing countries depend on the traditional medicinal system in terms of healthcare and holistic healing. Therefore, the quest for new and affordable anti-cancer developmental novelties are consistently escalating, one of which is the utilization of medicinal plants with their isolated bioactive compounds.

Two of the most used standardized assays in the field of anticancer developmental studies are the cytotoxicity

and genotoxicity assays. The aforementioned assays are performed to determine if biomaterials contain significant quantities of harmful extractables and their effect on cellular components. Specifically, for the cytotoxicity assay; the fertilization bioassay primarily uses sea urchin gametes that has been utilized by a variety of laboratories (Dinnel *et al.*, 1989). In terms of genotoxicity, within the biocompatibility tests available in the general field, the *Allium cepa* assays are of particular importance because it is used as an indicator of carcinogenicity (Ribeiro, 2008).

The Philippines is one of the Asian countries considered to house a diverse flora with numerous species alleged to possess curative properties. In the Bicol region, *Canarium ovatum* locally known as pili is a tropical tree native in the Philippines of the Burseraceae family. *Cocos nucifera* (L.), on the other hand, is a significant member of the family Arecaceae prevalently known as coconut. In terms of phytochemical compounds, the pili pulp is

composed of different glycerides, and saturated fatty acids (Intengan *et al.*, 1968). The kernel, as studied by Ragasa and colleagues (2013), contains different triterpenes and glycerols. Likewise, the *Cocos nucifera* water contains phytohormones which play a crucial role in the regulation of plant growth and exhibits antimicrobial activity, an antitumor potential, antioxidant, anti-inflammatory, antinociceptive and anti-neoplastic properties (Yong *et al.*, 2009).

Up to date, many phytochemicals are still being tested for their specific functions and deliveries and with these findings, the goal of isolating such compounds are the targets of today's experimental set-ups. To further elucidate the potential of the secondary metabolites of pili and coconut bark as potential anticancer agents, phytochemical screening, cytotoxicity and genotoxicity activities by using sea urchin fertilization assay and *Allium cepa* assay was done.

Materials and Methods

Collection and Preparation of Extracts

Canarium ovatum and *Cocos nucifera* bark samples were collected from Brgy. Tigbao, Casiguran Sorsogon, Philippines. The bark samples were dried under a shaded condition and further allowed to dry in oven at 40°C for 90 min to prevent the breakdown of bioactive components (Okwousa *et al.*, 2009). Completely dried bark samples were pulverized using mechanical mill and electrical blender. The powdered plant materials were soaked in 95% ethanol with 100 g : 200 ml ratio for 72 h. The sterilized containers that were used as storage jars were covered with aluminum foil to prevent penetration of light. The solution used for the extraction was filtered using Whatman® filter paper. The solvent was evaporated using rotary evaporator at 70°C with 70 rpm. The extracts obtained were distributed to petri plates to allow complete removal of solvent. After drying, the stock solution with a concentration of 500 mg/ml were serially diluted to different treatment concentrations for each of the plant extracts. For the *Canarium ovatum*, concentrations having 0.001 mg/ml, 0.01 mg/ml and 0.1 mg/ml were prepared, while 0.01 mg/ml, 0.1 mg/ml and 1 mg/ml were serially diluted *Cocos nucifera*. A preliminary screening using sea urchin fertilization assay using eight (8) concentrations was conducted to determine the half maximal inhibitory concentration of the two extracts.

Collection of Sea urchin (*Triplaneustes gratilla*)

Seven (7) male and eight (8) female sea urchins of mature age were collected from Sitio Matowoy, Brgy. Rawis, San Miguel Island Tabaco, Albay, Philippines. The preliminary checking of the sea urchins to determine their sexes were conducted before bringing it to the laboratory for the experiment proper. Collected sea urchins were transported to the laboratory of Bicol University Tabaco Campus, Tabaco Albay and the collected test subjects were placed immediately in an aerated tank with filtered sea water.

Sea Urchin Fertilization Assay

Sea urchins were injected with 2.0 ml of 0.5M Potassium chloride (KCl) through their peristomeal membrane to induce active shedding of gametes. The released gametes were collected in a small beaker with filtered sea water. Egg and sperm were identified based on its color and observed under light microscope for confirmation. A preliminary observation was conducted to select the viable egg cells based on the appearance of a prominent nucleus. Prior to fertilization, 2 ml of each concentration of the bark extracts were added to 6 well-plate with 3ml filtered seawater. 1% DMSO (Dimethyl sulfoxide) was used as the control group. A 0.2 ml sperm suspension was added to each well and allowed for 10 min exposure. After the sperm exposure, a 0.2 ml of egg suspension was added. The fertilization process was allowed to set for 20 min before fixation with 10% formalin. Successful fertilization was noted by evaluating the formation of vitellin membrane. Three replicates were done for each treatment. A total of 100 cells or zygotes were counted for each treatment to get the percentage of normal cells. The IC50 value of the extracts to inhibit fertilization in sea urchin was recorded in order to elucidate the concentrations to be used. The formula is as follows:

$$Y = \text{Low CI} + \frac{(\text{High CI} - \text{Low CI})}{1 + 10^{-(\text{Log IC}_{50} - X)}}$$

where 'Low CI' represents the minimum cell index values, 'High CI' represents the maximum cell index values, Y is the cell index, and X is the log of concentration (M) (Hazekawa *et al.*, 2019).

Allium cepa Test

The following procedures is a standard technique used to test genotoxicity using the *Allium cepa* test adopted from Ribeiro and colleagues (2016). *Allium cepa* bulbs were obtained from Daraga and Legazpi Albay, Philippines public market. The scales of the bulb were

removed, without disturbing the primordials. A total of 30 bulbs were grown in tap water for 3 days. After the pre-treatment with water for root growth, the bulbs were placed in different concentrations (0.01, 0.1 and 1 mg/ml) of *Canarium ovatum* and *Cocos nucifera* extracts. Tylenol solution was used as positive control while tap water was used as negative control. Three replicates were also done for each treatment. After 2 days of exposure to extracts, the root tips from each bulb were harvested and fixed in Carnoy's solution (1:3 Acetic acid : alcohol) for 1 h before proceeding with the slide preparation and analysis.

Slide Preparation and Analysis

After the pre-treatment, the root tips were rinsed a few times with distilled water. They were hydrolyzed with 5M HCl solution at room temperature for 5 min. After hydrolysis, the roots were squashed using a scalpel and stained with aceto-orcein (Sigma-aldrich) for 2 min. The slides were viewed using an Optical Microscope to evaluate mitotic phases and chromosomal aberrations. A total of 1000 cells per treatment were recorded. The mitotic index was calculated using the following equation:

$$\text{Mitotic Index} = \frac{\text{number of dividing cells}}{\text{total number of cells}} \times 100$$

The percent aberration was calculated using the following equation:

$$\text{Percent aberration} = \frac{\text{number of aberrant cells}}{\text{total number of cells}} \times 100$$

Phytochemical Analysis

Detection of Alkaloids. Dragendorff's test: 1 ml of extract was added with few drops of Dragendorff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids (Prashant Tiwari et al., 2011).

Detection of Diterpenes. Copper acetate test: 1ml of extract was dissolved in water and added with 10 drops of copper acetate solution, formation of emerald green colour indicated presence of diterpenes (Godghate, 2012).

Detection of Flavonoids. Alkaline Reagent Test: 1 ml of extract was added with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicated the presence of flavonoids (Prabhavathi, 2016).

Detection of Phenols and Tannins. Ferric Chloride Test: 1 ml of extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenols and tannins.

(Prashant Tiwari et al., 2011).

Detection of Saponins. Froth Test: 1 ml of extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 min. Formation of a layer of foam indicated the presence of saponins (Prashant Tiwari et al., 2011).

Detection of Terpenoids. Salkowski Test: 1ml of extracts was added with a few drops of chloroform. Then, a few drops of concentrated sulphuric acid (H₂SO₄) was carefully added to form a layer. A reddish brown coloration of the interface indicated the presence of terpenoids. (Edrah et al., 2016).

Statistical Analysis

The data for cytotoxicity and genotoxicity were analyzed using IBM SPSS Software v. 20. Values of the mitotic indexes and percent aberrations were analyzed and expressed as mean ± SEM. Differences between the treated and control groups were analyzed and compared using one-way analysis of variance (ANOVA), followed by Tukey HSD to determine their level of significance. Differences at p < 0.05 were considered statistically significant. The IC50 value of the extracts to inhibit fertilization in sea urchin was computed using the Graph Pad Prism v. 7.0 statistical software.

Results and Discussion

The present study was conducted to evaluate the cytotoxicity and genotoxicity of the bark extracts of *Canarium ovatum* and *Cocos nucifera* in sea urchin embryos and *Allium cepa* root tips, respectively. A total of two thousand four hundred (2, 400) sea urchin eggs and thirty-thousand (30,000) onion root cells were observed throughout the duration of the study.

Inhibitory concentration (IC50) determination

The treatments for the bark extracts were determined by range-finding test performed in the preliminary investigation of the study. During the preliminary test, concentrations ranging from 0.1 mg/ml to 0.8 mg/ml of both extracts were used to determine the final extract concentration to be used. For the *C. ovatum* bark extract as shown in Figure 1, the results showed that the range between 0.01 mg/ml and 0.8 mg/ml were the concentrations to be subjected to definitive testing. However, due to the high inhibitory activity of the extract, the concentration was reduced to 0.001 mg/ml, making

it the lowest concentration and 0.1 mg/ml the highest concentration. The generated IC₅₀ value for the *C. ovatum* extract is 0.54 mg/ml. Therefore, concentrations 0.001, 0.01 and 0.1 mg/ml of the extract were used in the study. For the *C. nucifera* bark extract, the results showed that the range between 0.01 mg/ml and 1 mg/ml were the concentrations to be subjected to definitive testing. The generated IC₅₀ value for the *C. nucifera* extract is 0.20 mg/ml. Therefore, concentrations of 0.01, 0.1 and 1 mg/ml of the extract were tested in the study. The concentrations generated from the preliminary test were also used for the genotoxicity screening. The concentrations used for both extracts in the genotoxicity screening were equal: 0.01, 0.1 and 1 mg/ml concentrations, respectively.

IC₅₀ of *C. ovatum*

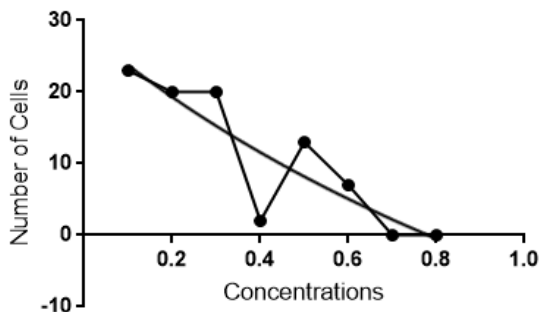


Figure 1. Fertilization inhibition curve of *Canarium ovatum* crude bark extract. IC₅₀ computed by range-finding test.

Inhibitory concentration (IC₅₀) determination

The concentrations of the *C. ovatum* bark extracts presented inhibitory activities on the fertilization of sea urchin eggs. The results indicate that the 0.001 mg/ml, 0.01 mg/ml and 0.1 mg/ml concentrations showed significant reductions in the percentage of fertilized egg compared with the control. Figure 3 shows that the 0.001 mg/ml concentration inhibited 76% of the sea urchin egg population. The 0.01 mg/ml concentration inhibited 79% of the sea urchin egg population and the 0.1 mg/ml concentration inhibited 86% of the sea urchin egg population, indicating the presence of possible cytotoxic compounds. For the concentrations of the *C. nucifera* bark extracts, the 0.01 mg/ml and 0.1 mg/ml concentrations, showed no significant difference as compared to the control. But as for the 1 mg/ml concentration, significant difference was observed as compared to the control. Figure 4 shows that the 0.01

IC₅₀ of *C. nucifera*

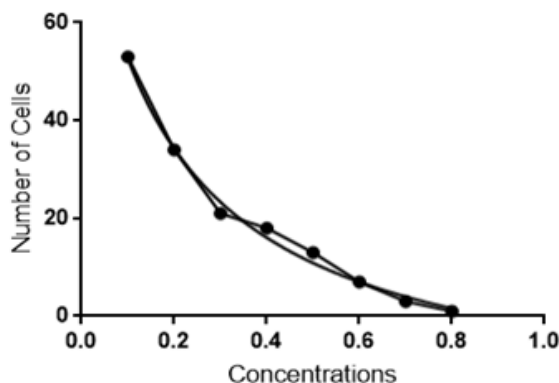


Figure 2. Fertilization inhibition curve of *Cocos nucifera* crude bark extract. IC₅₀ computed by range-finding test.

mg/ml concentration inhibited 5% of the sea urchin egg population. The 0.1 mg/ml concentration inhibited 9% and the 1 mg/ml concentration, inhibited 15% of the sea urchin egg population therefore indicating that there was no significant inhibition exhibited by the *C. nucifera* extract against the sea urchin egg fertilization. The effect of the extract on sea urchin fertilization may indicate an activity related to the inhibition of cellular motility of the sea urchin sperm cells. The result of the phytochemical analysis of the *C. ovatum* crude bark extract as presented. Table 1 shows parallel correlation with the findings that

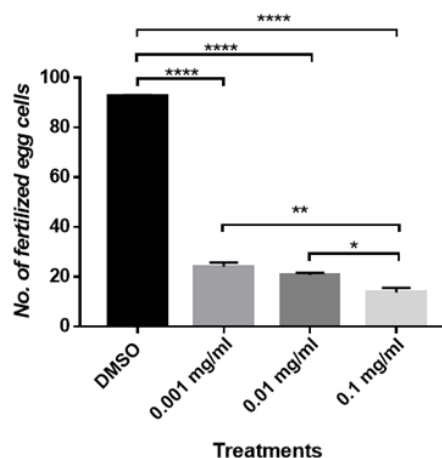


Figure 3. Number of fertilized sea urchin eggs exposed to different concentrations of *Canarium ovatum* crude bark extracts compared with control. Values are expressed as mean ± SEM. Significance of difference *p < 0.05 **p < 0.01 ****p < 0.0001

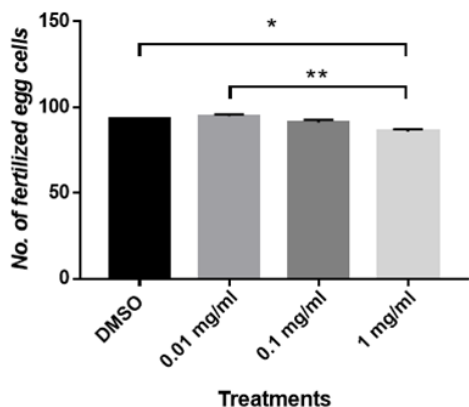


Figure 4. Number of fertilized sea urchin eggs exposed to different concentrations of *Cocos nucifera* crude bark extracts compared with control. Values are expressed as mean \pm SEM. Significance of difference * $p < 0.05$ ** $p < 0.01$

exhibited the said inhibiting activity against the egg cells at $p < 0.0001$ while the findings of the phytochemical results of the *C. nucifera* crude bark extracts show minimal to no presence of some bioactive compounds namely flavonoids, saponins, tannins, phenols, diterpenes and terpenoids which are known to exhibit antiproliferative and anticancer potentials.

Genotoxic activity of *C. ovatum* and *C. nucifera* extracts

The concentrations of the *C. ovatum* bark extracts

exhibits a dose-dependent activity of inhibiting cell division in onion root cells. Increasing concentrations of *C. ovatum* extracts in terms of mitotic index, decreases the capacity of the cells to undergo mitosis and arresting it at the interphase stage as shown in Figure 5. The positive control, inhibited 77% percent of the mitotic capacity of the cells. The 0.01 mg/ml and 0.1 mg/ml concentrations inhibited 80% of the mitotic capacity of the cells while the highest concentration at 1 mg/ml inhibited one hundred (100%) percent of the mitotic capacity of the cells. The positive control was significantly different from the highest treatment concentration at $p < 0.001$. This indicates that it has very high capability of inducing decreased cell division compared to the control.

Also, the results from the 0.01 mg/ml and 0.1 mg/ml concentration exhibits that the growth and development of the exposed test subject have been affected by the plant extracts. In terms of percent aberration, increasing concentrations of *C. ovatum* extracts increases the frequency of aberrations in the cells. The 0.1 mg/ml concentration showed a high percentage of aberration which is statistically different to the positive control at $p < 0.001$ and $p < 0.01$, respectively. This indicates that the concentration exhibited a high toxic activity as compared with the positive control. The 1 mg/ml concentration exhibited no aberrant percentage because all cells treated with this concentration were arrested at the interphase stage and no aberrant cells were observed and recorded. In terms of mitotic index, the concentrations of *C. nucifera* bark extracts did not show any significant difference as compared to the positive control. Extracts do not induce high cell proliferation even if the concentration increases

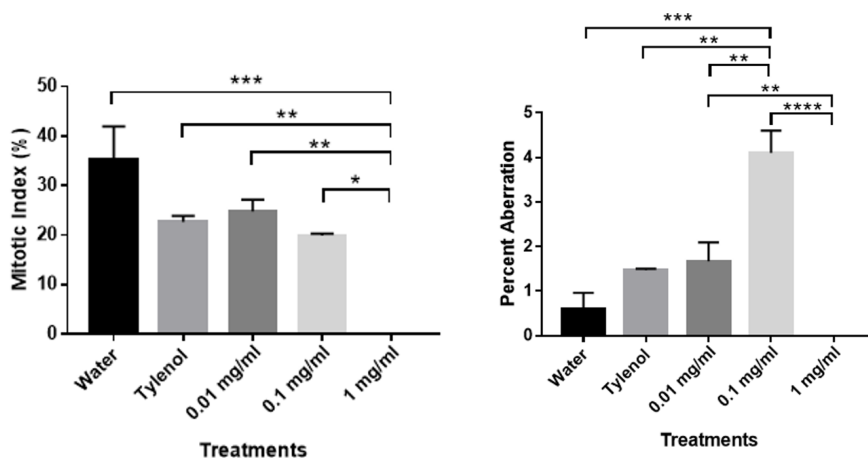


Figure 5. Recorded percentage values of Mitotic Index and Percent aberration of *Canarium ovatum* crude bark extracts. Values are expressed as mean \pm SEM. Significance of difference * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ **** $p < 0.0001$

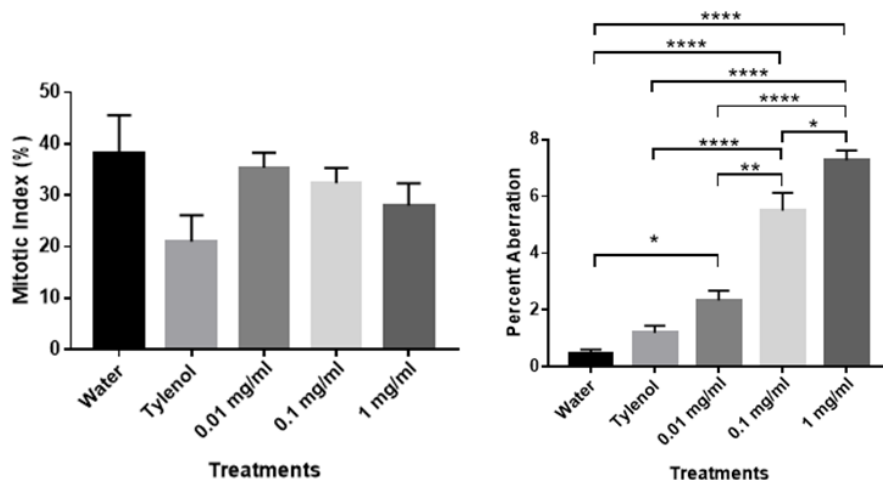


Figure 6. Recorded percentage values of Mitotic Index and Percent aberration of *Cocos nucifera* crude bark extracts. Values are expressed as mean \pm SEM. Significance of difference * $p < 0.05$ ** $p < 0.01$ **** $p < 0.0001$.

as shown in Figure 6. As compared to the positive control treatment, the positive control still has the higher capacity of inducing mitotic inhibition compared to other concentrations, indicating that the extract does not present high toxicity activity. In terms of percent aberration, the concentration of *C. nucifera* bark extracts specifically 0.01 mg/ml and 1 mg/ml showed very high statistical difference to both negative and positive controls at $p < 0.0001$. This indicates that the 0.1 mg/ml and 1.0 mg/ml concentrations exhibited higher toxicity activity than the positive control.

Phytochemical Screening

The phytochemical analysis has revealed the presence of various compounds in both extracts tested. In *C. ovatum* crude bark extract, compounds such as flavonoids, phenols and tannin were found abundant with traces of saponins. In *C. nucifera* crude bark extract compounds such as saponin was found abundant with traces of flavonoids, phenols, tannins, diterpenes and terpenoids as shown in Table 1.

Discussion

In the study of Cajuday and co-workers (2017), it was elucidated that the medium and high concentrations of COPE (*Canarium ovatum* pulp extract) exhibited significant reductions in the percentage of sea urchin fertilized egg cells. Similar results are observed from the study of Chattopadhyay and colleagues (2005). Saponins and terpenes which are widely available in both plants in this study are reported to have spermicidal activity. Saponin which is found in both plants is found to have significant sperm-immobilizing activity. In the treatment of sea urchin eggs with quercetin, it was found to have blocked fertilization without significantly affecting sperm-egg binding (Eckberg, 1983). The effects of quercetin on gamete fusion may be general. Research found that quercetin blocks fertilization in *Spisula* (Eckberg, 1982) and *Chaetopterus*. Fluorescein dyes have been suggested to inhibit membrane fusion in sea urchins (Carroll & Levitan, 1978). In the study of Gutierrez (2016), the phytochemical analysis of *C. papaya* showed that

Table 1. Phytochemical screening results of *Canarium ovatum* and *Cocos nucifera* crude bark extracts

Plant species	Alkaloids	Diterpenes	Flavonoids	Phenols	Saponins	Tannins	Terpenoids
<i>Canarium ovatum</i>	-	-	+++	+++	+	+++	-
<i>Cocos nucifera</i>	-	+	+	+	+++	+	+

terpenoids blocks mitosis by stabilizing tubulin polymers and thereby inhibiting disassembly of microtubules in sea urchin eggs. Sea urchin embryogenesis was known to have well-defined developmental stages that demand microtubule function. Microtubules are extremely important cellular entity with a crucial role in shape maintenance, cell motility, intracellular transport and cell division. The phytochemical analysis of *A. macrophylla*, revealed the presence of triterpenes, flavonoid and tannins which showed high efficacy for inhibiting sea urchin sperm motility assessed by the microscopic methods (Saha, et al. 2007). In the study of Zhou and colleagues (2012), it was shown that tannin-protein reaction could congeal seminal plasma protein *in vivo* to inhibit mobility of semen, react with sperm membrane protein for decreasing the stability and integrity and deactivate the metabolic enzyme (both the metal center and protein) to interrupt the energy-supplying chain.

Furthermore, in the study of Au and colleagues (2000), convolution of the plasma membrane was observed in the sea urchin and mussel spermatozoa after phenol treatment (500 ppm). In addition, the noticeable changes in midpiece shape of sea urchin sperm may affect the balance of swimming sperm, thereby leading to decline in sperm motility. Killian and colleagues (1985) noted that Aphidicolin, tetracyclic diterpene antibiotic isolated from a fungus, has now been shown to block DNA replication in fertilized eggs of four sea urchin species and aphidicolin-treated fertilized sea urchin egg, in which RNA synthesis, protein synthesis and many other cytoplasmic processes proceed in the absence of chromosome replication and mitosis, is, in these regards, functionally equivalent to the differentiated non-cycling cell. Additionally, it was found that dolastanes, a type of diterpene compound inhibited Na⁺ K⁺ pathway, making it impossible for the sperm and egg to achieve syngamy because the sperm is motile when it is activated by ATPase at an alkalized environment (Ramirez et al., 2013).

The types of aberrations recorded are exhibited in Figure 5 based from the studies of Mohammed and colleagues (2015) and Ping and colleagues (2012). The *Allium cepa* test has shown good correlation with other test systems, both in general toxicity (growth) and in genotoxicity (chromosome aberrations) (Fiskesjo, 1993). As mentioned above, mitotic indexes (MI) can be used as a parameter of cytotoxicity. Mitotic indexes lower than the negative control may indicate that the growth and development of exposed organisms have been affected by test compounds. On the other hand, MI's above those of the negative control are result of the induction of increased cell division, which may characterize an event

detrimental to cells, leading to uncontrolled proliferation and even tumor formation. The increase or decrease in MI can be an important indicator to monitor pollution levels in affected environments, especially those contaminated with potentially toxic and cytotoxic compounds (Hoshina, 2002). Smaka-Kincl and colleagues (1996) reported that a decreased mitotic index of meristematic cells of *A. cepa* may be considered a reliable method to determine the presence of cytotoxic compounds in the environment, and thus be a suitable test for monitoring pollution levels (Caritá & Marin-Morales, 2008). As presented in Table 1, the phytochemical analysis of the two bark extracts presented a parallel correlation with the compounds responsible for such activities. These bioactive compounds are flavonoids, saponins, tannins, phenols, diterpenes and terpenoids that are known to possess antiproliferative, anti-genotoxic and anti-mutagenic potentials, as based from the articles obtained. In the study of Moscoso and colleagues (2015), the observed anti-proliferative effect of the extracts (*Clusia latipes*) on selected cancer cells could be attributed to the presence of hesperidin, a flavonoid compound, as well as the two triterpenes isolated, friedelin (1) and friedolan-3-ol. Romero and co-workers (2002) demonstrated the capacity of rutin, a flavonoid compound, to inhibit the proliferation of LNCaP cells (prostate cancer) at 24, 48, 72 and 96 h of treatment. The results showed that rutin, at concentrations of 50 and 75 μ M, inhibited cell proliferation starting at 24 h of treatment. According to Woo and colleagues (2005), some flavonoids such as quercetin and genistein could inhibit cell proliferation by modulating the activity of cyclin-dependent kinases, key molecules in the regulation of the cell cycle. Flavonoids inhibit the growth of tumor cells by impeding progression of cells through the G₀-G₁ transition of the cell cycle, subsequently leading to apoptosis. It is possible that cyclins are also targets of rutin activity, as seen with other flavonoids, which could explain the anti-proliferative effect observed (Marcarini, 2009). In a study, flavonoids can be potentially harmful since some are mutagenic in bacterial and mammalian test systems. The genotoxicity, mutagenicity and enzymatic inhibition caused by exposure to tobacco leaves was probably mediated by the complex mixture of substances (nicotine, coumarins, traces of saponins, flavonoids and different inorganic elements) present in these leaves (da Silva et al., 2013).

From the review of the U.S. Environmental Protection Agency (EPA) in 2002, results from DNA damage assays are inconsistent, but they tend to show that phenol can cause sister chromatid exchanges. A study from Khalil and colleagues (1994), reported that diverse biological properties of saponins have been

reported. At high concentrations, saponins caused cell damage by disrupting the cell membrane or inducing apoptosis. Anti-tumor-promotion and growth inhibition of saponins on tumors or tumor cell lines were also reported (Berhow *et al.*, 1999). In the study of Buyukleyla and colleagues (2012), tannic acid showed a synergistic cytotoxic effect by decreasing the MI. In the study of Lin and colleagues (2014), Flavonoids (such as quercetin, kaempferol, and isorhamnetin) and terpenoids (including *ginkgolide A*, *ginkgolide B*, *ginkgolide C*, and *bilobalide*) are the two major classes of components found in *Ginkgo biloba*. It has been reported that some flavonoids, with variable chemical structures, target DNA topoisomerases and interrupt the process of DNA replication. Prolonged inhibition of topoisomerases leads to unrepaired DNA breaks, thus resulting in genotoxic effects such as multilocus deletions, chromosomal translocations, and loss of heterozygosity (Zhang *et al.*, 2015). The results show significant inhibition and toxicity of the plant extracts on the test samples.

Fruits and vegetables with flavonoids have been reported as cancer chemopreventive agents and several flavonoid derivatives are also reported to have antimutagenic activity (Kumar *et al.*, 2013). Quercetin, a bioflavonoid compound has been found to be a potent anticarcinogen against cancers of skin, colon, and mammary in rodents (Chung *et al.*, 2010). Plants containing phenols are described to have anticancer property by acting as an antioxidant that gives protection from the effect of oxygen radicals and reduces the activity of xanthine oxidase, an enzyme known to be involved in carcinogenesis (Tripoli *et al.*, 2005). One of the studied biological activity of tannin is its potential as anticarcinogen. In the study of Chung and colleagues (2010), tannin and its derived compounds promoted tumor inhibition in mice model. This activity of tannin can be attributed to its antioxidative property.

On the screening conducted for the activity of *Nauclea* bark extract, saponins were found to be one of

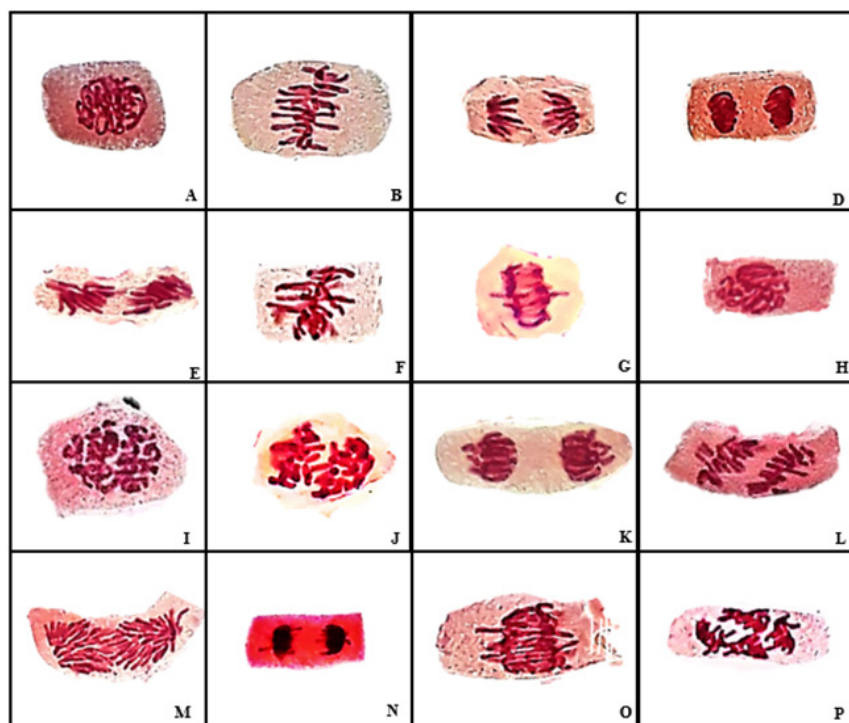


Figure 7. Micrographs showing chromosomal aberrations in *C. nucifera* extract-treated *Allium cepa* root cells. (A) Normal Prophase (B) Normal Metaphase (C) Normal Anaphase (D) Normal Telophase (E) Chromosomal bridge in anaphase (F) Sticky metaphase (G) Vagrant chromosome in anaphase (H) and (I) Disrupted prophase (J) C-mitosis (K) Sticky telophase (L) Spindle abnormalities (M) Chromosomal bridges and laggard (N) Sticky anaphase (O) Delayed anaphase (P) Diagonal metaphase. Stained with aceto-orcein stain; 400X

the most active bio-compound. The results of COMET assay indicated that the mixture of saponins could induce DNA damage on CHO cell lines thus having direct genotoxic effect (Liu *et al.*, 2011). There are a number of *in vitro* studies that shows the cytotoxic and genotoxic activities of terpenoids including diterpenes. Androgapholide, a diterpene, demonstrated inhibitory effects against the growth of hepatoma-derived Hep3B cells. Terpenoids were observed to induce apoptosis, inhibit cell proliferation and also modulation of multiple signal transduction pathways (Thoppil *et al.*, 2011). All of the discussed bioactive compounds were present in the plant extracts supporting the results generated from the different assays.

Conclusion

The quest for new and affordable anti-cancer developmental novelties has encouraged a rigorous discovery of alternatives. This study elucidated that *Canarium ovatum* bark extracts presented high cytotoxic activity causing an inhibition on the fertilization of sea urchin eggs. On the other hand, *Cocos nucifera* bark extracts presented low cytotoxic activity as manifested by the low number of inhibited fertilized eggs. As for the genotoxicity, *Canarium ovatum* bark extracts exhibited high anti-proliferative activity as compared to the *Cocos nucifera* bark extracts which only exhibited low anti-proliferative activity. Moreover, high percentage of chromosomal aberrations were observed in *Canarium ovatum* bark extracts while low percentage of chromosomal aberrations in *Cocos nucifera* were detected. Hence, the plant extracts from *Canarium ovatum* and *Cocos nucifera* can serve as alternatives for cancer treatment but it is recommended to use the said extracts in cancer cell lines to ascertain its anticancer efficiency.

Acknowledgments

The authors would like to extend their gratitude to the faculty of the Bicol University College of Science Department of Biology and to the faculty and administrative personnel of Bicol University – Tabaco Campus.

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