

## Antioxidant and Antimutagenic Activities of Ripe Bignay (*Antidesma bunius*) Crude Fruit Extract

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### Abstract

Bignay is a tropical fruit with known beneficial compounds. However, little literature has investigated its dual antioxidant-antimutagenic properties. In the current study, the ripe fruits of bignay were analyzed for total antioxidant capacity, lipid peroxidation inhibition, genotoxicity and antimutagenicity against methyl methanesulfonate in onion root meristematic cells to determine the antioxidant and antimutagenic activities. The fruit extract has lower antioxidant capacity and lipid peroxidation inhibition compared to L-Ascorbic Acid, but showed a dose-dependent activity. The fruit extract did not induce genotoxicity to DNA repair-deficient *Escherichia coli* PQ37 and reduced the chromosomal aberrations in onion root cells. The highest antioxidant and antimutagenic activities was observed at 1000µg/mL, comparable to 1000 µg/mL of L-Ascorbic Acid ( $p < 0.01$ ). The activities of bignay fruit extract is attributed to the net effects of all compounds. Over all, bignay fruit extract possesses a promising antioxidant-antimutagenic activity.

**Keywords:** antioxidant capacity, antimutagenic activity, bignay fruit extract, chromosomal aberrations, genotoxicity, lipid peroxidation inhibition

### Introduction

Bignay (*Antidesma bunius*), a member of family *Phyllanthaceae*, is gaining recognition as a rich source of bioactive compounds that exhibit notable pharmacologic properties. Several studies have reported that the fruit extract of this tropical fruit exhibited antibacterial properties (Lizardo, Mabesa, Dizon, & Aquino, 2015),  $\alpha$ -glucosidase inhibitory activities (Lawag, Aguinaldo, Naheed, & Mosihuzzaman, 2012), antidiabetic properties (El-Tantawy, Soliman, El-naggar, & Shafei, 2015), and notable antioxidant properties (Belina-Aldemita, Sabularse, Dizon, Hurtada, & Torio, 2013). Bignay fruits are typically eaten

raw or processed as tea, jelly, wine or jam in the Philippines, and the medicinal properties reported in previous studies can further extend the utilization of the ripe fruits as medicinal products.

In some studies, the bioactive compounds in plant extracts were reported to exhibit dual antioxidant and antigenotoxic properties. Badmus et al. (2013) reported that the leaves of *Holarrhena floribunda* exhibited antioxidant, antimutagenic and lipid peroxidation inhibition. These characteristics were also reported to be exhibited by *Phaseolus vulgaris* seeds (Cardador-Martinez et al., 2006) and *Anemopsis californica* (Del-Toro-Sanchez, 2014). Some plant extracts

may also exhibit genotoxic or cytotoxic activities which usually vary with the concentration of the extracts in different solvents (Aşkin Celik & Aslantürk, 2010; Celik, 2012).

Although bioactive compounds in plant extracts were emphasized to suppress oxidative damage that leads to genotoxicity, the effects of plant extracts against alkylating agents is less emphasized. The main effect of alkylating agents is the promotion of the formation of alkylated lesions to guanine and thymine (Mackay, Han, & Samson, 1994), which is relatively different compared to the DNA damage caused by oxidative processes. Methyl methanesulfonate (MMS), an alkylating agent, promotes methylation in the nucleophilic sites of nitrogenous bases leading to base modifications (Medeiros Mazzorana, Nicolau, Moreira, de Aguiar Amaral, & de Andrade, 2013).

The mutagenic properties of alkylating agents are currently utilized to treat cancer. Potentiating the mutagenic effects of alkylating agents may be beneficial in suppressing carcinogenesis, although the damage to normal cells may present a risk. Hence, the utilization of plant extracts as adjuvant in anticancer therapy using alkylating agents may have positive implications. As bignay has been previously reported to contain several compounds with antioxidant properties (Bukthup & Samappito, 2011), the antimutagenic activities against alkylating agents may increase its uses. Despite its several medicinal properties, the antimutagenic property of bignay fruit extract against methyl methanesulfonate (MMS) has not been previously reported elsewhere.

Hence, this study investigated the antioxidant activity, genotoxicity and antimutagenic effects of crude bignay fruit extract against the mutagenic properties of MMS, an alkylating agent, using an *in vivo* plant model, *Allium cepa* roots.

## Materials and Methods

### Chemicals and Reagents

Methanol, deionized water, gallic acid, and quercetin were purchased from Merck Millipore, Germany. Sodium nitrite ( $\text{NaNO}_2$ ), Sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), methylmethanesulfonate (MMS), Sodium acetate trihydrate ( $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$ ), Potassium chloride (KCl),

Aluminum chloride hexahydrate ( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ), Ferrous sulphate ( $\text{FeSO}_4$ ), Sodium phosphate ( $\text{Na}_3\text{PO}_4$ ), ammonium molybdate, Sodium hydroxide (NaOH), L-Ascorbic acid and Sulfuric acid ( $\text{H}_2\text{SO}_4$ ) were purchased from Sigma Aldrich, Singapore. The SOS Chromotest Kit was purchased from Environmental Bio-detection Products Inc. (EBPI), Ontario, Canada. All reagents used in the study were of analytical grade. The chemicals and reagents were purchased from specific chemical suppliers to ensure the quality of the reagents and materials used in the study.

### Preparation of Bignay Fruit Extract

Two kilograms of fully ripe bignay fruits were collected from Bagulin, La Union and transported immediately to the Saint Louis University Natural Science Research Unit (SLU-NSRU), Baguio City. The plant sample was authenticated at Saint Louis University Herbarium and assigned with Herbarium Accession Number SLUH No. 23. The undamaged ripe fruits were sorted then washed with distilled water twice. The fruit pulp was separated from the seed, placed in a plastic pan, air dried for two days and oven-dried at  $90^\circ\text{C}$  for three days. Dried fruit pulps were ground finely using a blender. In a clean Erlenmeyer flask, 100 grams of the ground fruit pulp was extracted using 95% methanol solution for 72 hours at room temperature ( $22^\circ\text{C}$ ). The resulting extract was filtered twice with Whatman 1 filter paper, and then evaporated using a rotator evaporator at  $45^\circ\text{C}$ . The residue was collected in an amber bottle and stored in the ultra-low freezer ( $-20^\circ\text{C}$ ) until used.

### Determination of Total Phenolic Content

The determination of total phenolic content was described in the methodology of Saeed *et al* (2012) using gallic acid as a reference standard. In a clean test tube, 100mg of the collected residue was dissolved in 100mL deionized water to prepare a 1mg/mL stock solution of the fruit extract. In separate test tubes, 1mL of the solutions were mixed with 1 mL of Folin-Ciocalteu reagent, followed by 5mL of 7%  $\text{Na}_2\text{CO}_3$  solution and 13 mL of deionized water. The tubes were covered with Aluminum foil and set aside

for 90 minutes. After the colour reaction, the absorbance of the samples was determined using a spectrophotometer at 750 nm against a reagent blank (solvent + Folin-Ciocalteu reagent), and the total phenolic content was calculated using a calibration curve. The concentration was expressed as mg gallic acid equivalents per 100g DW of the extract (mg GAE/100gDW).

#### *Determination of Total Flavonoid Content*

The methodology for estimating the total flavonoid content was also adapted from the methodology of Saeed *et al* (2012) with modifications. The total flavonoid content of bignay was determined by mixing 0.3mL of the extract solution with 3.4mL of 30% methanol, 0.15mL of NaNO<sub>2</sub> solution (0.5N), and 0.15mL of AlCl<sub>3</sub>·6H<sub>2</sub>O (0.3M). After five minutes, 1mL of NaOH solution (1M) was mixed thoroughly. The absorbance capacity of the solutions was measured using a spectrophotometer at 506 nm against a reagent blank and the total flavonoid content was calculated using a calibration curve. The concentration was expressed as mg quercetin equivalents per 100g DW of the extract (mg QE/100g).

#### *Determination of Total Monomeric Anthocyanin Content*

The total monomeric anthocyanin content of the sample was determined using pH differential method described in the study of Lee *et al* (2008). A pH 1.0 buffer solution was prepared using 1.86g KCl in 1000 mL distilled water (analytical grade). A pH 4.5 buffer solution was prepared by dissolving 54.43g of CH<sub>3</sub>COONa·3H<sub>2</sub>O in 1000mL analytical grade water. The pH of the two buffer solutions were adjusted using concentrated HCl. In separate test tubes, 0.5 mL of the stock solution was mixed with 5 mL of each of the buffer solutions. After the colour reactions, the absorbances of the mixtures were obtained spectrophotometrically at 520 nm and 700nm. The values were used to obtain the value of A in the equation:

$$\text{TAC} \left( \frac{\text{mg}}{\text{L}} \right) = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\epsilon \times l}$$

Where:

$$A = (A_{520} - A_{700})_{\text{pH } 1.0} - (A_{520} - A_{700})_{\text{pH } 4.5}$$

Total monomeric anthocyanin content was expressed as mg/L cyanidin-3-glucoside equivalents (C-3-G eq, MW of cyanidin-3-O-glucoside = 449.2 g·mol<sup>-1</sup>,  $\epsilon$  = 26,900 L·cm<sup>-1</sup>·mol<sup>-1</sup>, DF = 10, and l = 1 cm). The final unit was expressed as mg total anthocyanin content per 100g dry weight of the extract (TAC/100gDW).

#### *Determination of Total Antioxidant Capacity*

A modified phosphomolybdate method was adapted from the article of Shahwar *et al* (2012) to estimate the antioxidant capacity of bignay fruit extract. In separate test tubes, 1mL of fruit extract solutions (125µg/mL to 1000µg/mL) was mixed with 1mL of reagent solution (0.6M H<sub>2</sub>SO<sub>4</sub>, 28mM Na<sub>3</sub>PO<sub>4</sub>, and 4mM ammonium molybdate). The tubes were sealed with parafilm, incubated in a water bath at 95°C for 90 minutes, and then cooled to room temperature. The absorbance was measured at 765nm against a reagent blank composed of reagent solution without the fruit extract. L-Ascorbic Acid was used to estimate the total antioxidant capacity and expressed as mg/L L-Ascorbic Acid Equivalents.

#### *Lipid Peroxidation Inhibition Assay*

A modified method described by Wang and Wang (2008) was used to assess the lipid peroxidation inhibition of bignay fruit extract. A volume of 0.50mL of buffered egg yolk homogenate was mixed with 2mL of thiobarbituric acid reagent (20% glacial acetic acid and 0.67% 2-thiobarbituric acid in 0.25N HCl solution). The mixture was mixed with 0.5mL of the prepared bignay fruit extract solutions. Next, 0.2mL of 0.15mM FeSO<sub>4</sub>·7H<sub>2</sub>O was introduced to induce lipid peroxidation, then heated in a water bath for 30 minutes, cooled, and centrifuged at 2000g for 5 minutes. The supernatant was collected and transferred to a cuvette and the absorbance was obtained spectrophotometrically at 532 nm using the reagent as the blank solution. The negative control was the reagent without the plant extract while the positive control was L-Ascorbic Acid. The % inhibition was computed using the formula:

$$\% \text{ Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

### Genotoxicity Assay

The genotoxicity of bignay fruit extract was evaluated using SOS Chromotest (EBPI, Canada) and with *Escherichia coli* PQ37 as test organism. The assay was based on SOS gene complex repair promoter region expression that activates the SOS genes linked to the  $\beta$  gal gene, which in turn produces the  $\beta$ -galactosidase enzyme, indicating bacterial cell viability (Quintero *et al*, 2012). SOS Induction Factor (SOSIF), the indicator of genotoxicity, was obtained using the Excel Template provided with the SOS Chromotest kit. A substance is not genotoxic if SOSIF is <1.5, inconclusive if SOSIF is between 1.5 and 2.0, and genotoxic if SOSIF is > 2.0 and a clear concentration-response relationship was observed. The SOSIF of bignay fruit extract was compared to 4-nitroquinoline-1-oxide (4-NQO), a known genotoxicant. The procedure was performed aseptically in triplicate.

### Antimutagenicity Assay

The Onion (*Allium cepa*) Chromosomal Aberration Assay was adapted from the study of Ping *et al* (2012). The method was modified to assess the antimutagenicity of bignay fruit extract against methylmethanesulfonate (MMS).

#### Pre-treatment

Medium-sized onion (*Allium cepa* L.) bulbs (15 to 20 grams) were obtained from Rosario, La Union. The bulbs were washed with distilled water, followed by surface disinfection using 10% H<sub>2</sub>O<sub>2</sub> solution. The roots were removed to allow new root growth. The selected onion bulbs were immersed in deionized water for 72 hours until new roots are about 2 – 3 cm long and then transferred to the following solutions:

- |                      |  |
|----------------------|--|
| Positive Control:    | Methyl methanesulfonate (10mg/mL, MMS)       |
| Experimental groups: | 1000µg/mL bignay fruit extract + 10mg/mL MMS |

500µg/mL bignay fruit extract + 10mg/mL MMS

250µg/mL bignay fruit extract + 10mg/mL MMS

125µg/mL bignay fruit extract + 10mg/mL MMS

Comparison group: 1000 µg/mL L-Ascorbic Acid + 10 mg/ mL MMS

Negative control: Deionized Water

After 48 hours of treatments about 2 – 3 cm of the roots were collected, rinsed thrice using distilled water, and fixed for 24 hours in Carnoy's fixative (1:3 glacial acetic acid: ethyl alcohol) for 24 hours). After fixing, the roots were rinsed with distilled water thrice then preserved in 70% ethanol solution at 4°C until used for slide preparation.

### Slide Preparation and Microscopic Observation

The roots were rinsed with distilled water thrice to remove the ethanol solution then hydrolyzed in HCl (1N) at 70°C for 5 minutes. A sterile scalpel was used to cut 2 mm of the root tips, which were then carefully placed in a microscope slide. The root tips were stained with orcein solution (2% orcein in 45% acetic acid) for two minutes. After staining, the root tips were squashed with a blunt metal tip and stained again for another two minutes. A clean cover slip was lowered carefully, sealed with a clear nail polish, labeled appropriately and stored at 4°C until ready for examination. A total of 10 slides per treatment were prepared. The slides were observed using a light microscope at 600 to 1000x magnification. The chromosomal aberrations were counted and documented using a digital camera and a minimum of 5000 root tip cells per treatment were evaluated using randomly selected fields of view. Five replicates were performed to obtain the mean ± standard deviation. The following data were obtained:

$$\text{Mitotic Index (MI)} = \frac{\text{Number of cells in Mitosis}}{\text{Total Number of Cells Observed}} * 100$$

$$\%DR = \frac{[\text{MI Aberrant Cells (MMS)} - \text{MI Aberrant Cells (Treatment Group)}]}{\text{MI Aberrant Cells (MMS)} - \text{MI Aberrant Cells (Deionized Water)}} * 100$$

The % damage reduction (%DR) reflects the antimutagenic ability of the plant extract against methyl methanesulfonate.

### Statistical Tests

Data were presented in tables and figures using mean  $\pm$  standard deviation, with three to five replicates per treatment. One way analysis of variance (ANOVA) with post hoc Fisher LSD test was utilized to determine the significant differences of the means in the different assays. The observed chromosomal aberrations in all treatments were reported as frequency. Statistical analysis was performed using SPSS 20.0 for Windows at  $\alpha=0.01$ .

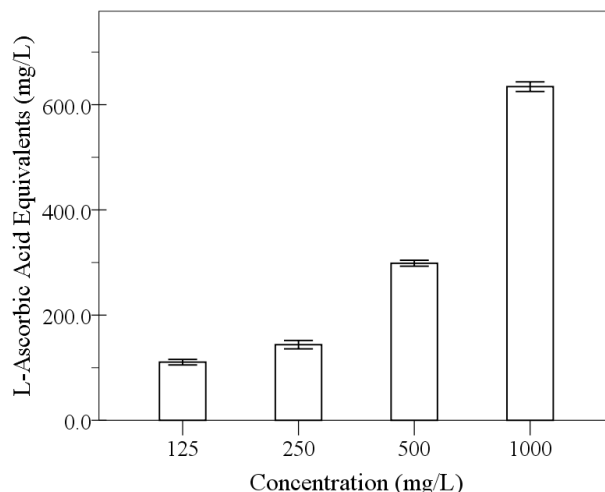
## Results and Discussion

### Characterization of the Fruit Extract

The concentration of phenolic compounds, flavonoids and monomeric anthocyanins per 100g DW of bignay fruit extract is shown in Table 1. Phenolic compounds had the highest concentration in the fruit extract, followed by flavonoids and monomeric anthocyanins.

### Total Antioxidant Capacity

Based from the results shown in Figure 1, the total antioxidant capacity expressed as L-Ascorbic Acid Equivalents (mg/L) increases as the concentration of bignay fruit extract increases. This suggests that the relative activity of bignay fruit extract can be expressed as relative activity of L-Ascorbic Acid. It can be inferred that L-Ascorbic Acid had relatively higher total antioxidant capacity than bignay fruit extract.



**Figure 1.** Total Antioxidant Capacity of Bignay Fruit Extract

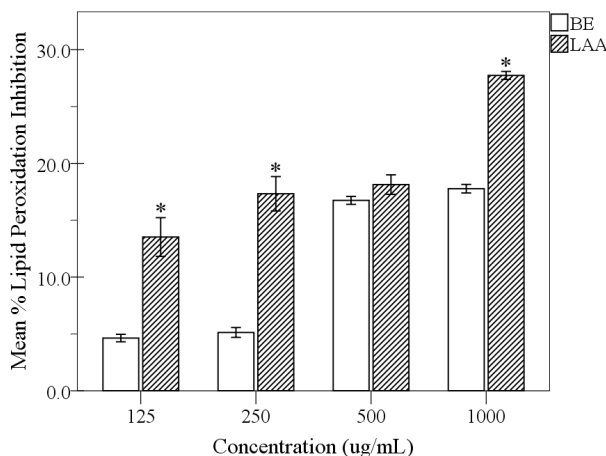
**Table 1.** Phenolic, Flavonoid and Anthocyanin Content of Bignay

Metabolites	Concentration (mg/100g DW of extract)
Total Phenolic Content	1978.38 $\pm$ 39.06* GAE
Total Flavonoid Content	1526.7 $\pm$ 2.30* QE
Total Monomeric Anthocyanin Content	131.42 $\pm$ 1.41* C3GE

\*mean of five replicates

**Lipid Peroxidation Inhibition**

The activity of bignay fruit extract to inhibit lipid peroxidation in egg yolk homogenate was concentration-dependent, similar to the activity of L-Ascorbic Acid (Figure 2). The activity of L-Ascorbic Acid was significantly higher compared to bignay fruit extract although the mean % inhibition of bignay fruit extract was comparable to L-Ascorbic Acid at 500 µg/mL ( $p < 0.01$ ). It can be noted, too, that the lipid peroxidation inhibition of bignay fruit extract was comparable at 500µg/mL and 1000µg/mL, indicating that the maximum lipid peroxidation inhibition may have been reached at this range of concentration.

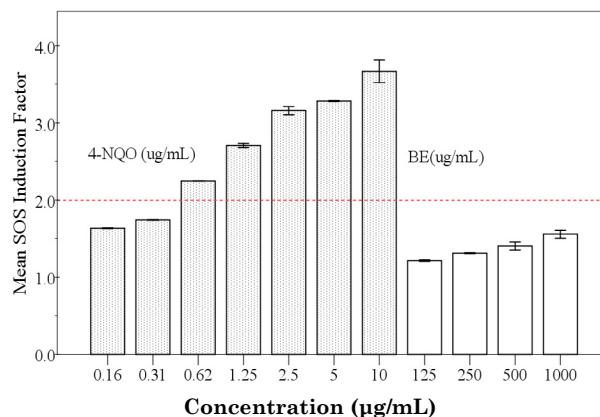


**Figure 2.** Lipid Peroxidation Inhibition of Bignay Fruit Extract and L-Ascorbic Acid

\*Indicates significant difference within the same concentration ( $p < 0.01$ )

**Genotoxicity in SOS Chromotest Assay**

Based from the data shown in Figure 3, bignay fruit extract was not genotoxic to *E. coli* PQ37 even at a concentration of 1000 µg/mL per assay while 4-nitroquinoline-1-oxide (4-NQO) was genotoxic at  $\geq 0.62 \mu\text{g} \cdot \text{mL}^{-1}$  per assay. The data suggests that the fruit extract does not induce primary DNA damage, although the SOS Induction factors of the extract shows a dose-dependent increase as the concentration of the extract increases.



**Figure 3.** Genotoxicity of Bignay Fruit Extract Compared to 4-NQO

\*BE = bignay extract, 4-NQO – 4-nitroquinoline-1-oxide

**Antimutagenicity Against MMS in Onion Root Cells**

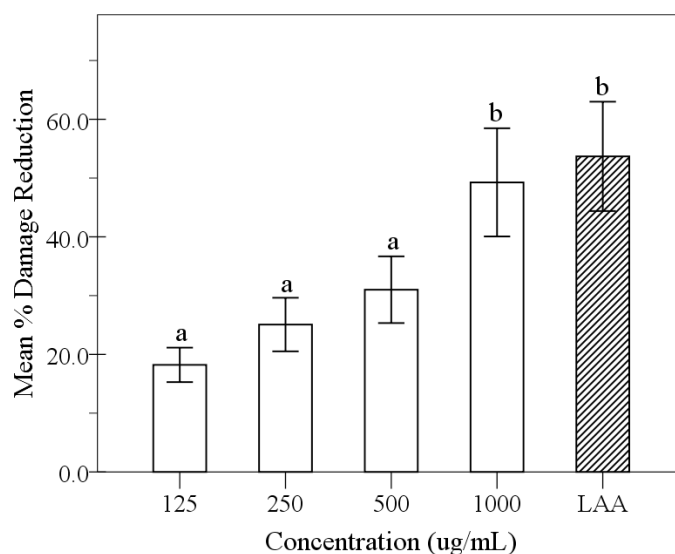
Table 3 summarizes the mitotic indices of onion root cells treated with MMS, bignay extract + MMS, L-Ascorbic Acid + MMS and deionized water. Bignay fruit extract seems to protect the onion root tip cells from the damaging effects of MMS based from the decreasing mitotic indices of aberrant cells. The mitotic indices of root cells treated with bignay fruit extract + MMS was higher compared to the cells treated with MMS only. Onion root cells in the negative control (deionized water) and L-Ascorbic acid + MMS had higher mitotic indices for non-aberrant cells but lower mitotic indices for aberrant cells. Figure 4 shows that the mean % damage reduction of by bignay fruit extracts when mixed with MMS was lower compared to the mean % damage reduction of L-Ascorbic Acid. This means that bignay fruit extract and L-Ascorbic Acid possess antimutagenic activities against MMS. Statistically, a 1000µg/mL of bignay fruit extract was comparable to the antimutagenic property of 1000µg/mL of L-Ascorbic Acid ( $p < 0.01$ ). The microscopic features of non-aberrant and aberrant cells are presented in Figure 5.

Fruit extracts and fruit juices have been shown to exhibit antioxidant and antimutagenic activities against alkylating agents (Franke, Pra, Erdtmann, Henriques, & da Silva, 2005; Melo-Cavalcante, Picada, Rubensam, & Henriques,

**Table 3.** Cytogenetic effects of MMS, bignay fruit extract (BFE) +MMS and L-AA + MMS in *Allium cepa* meristematic cells

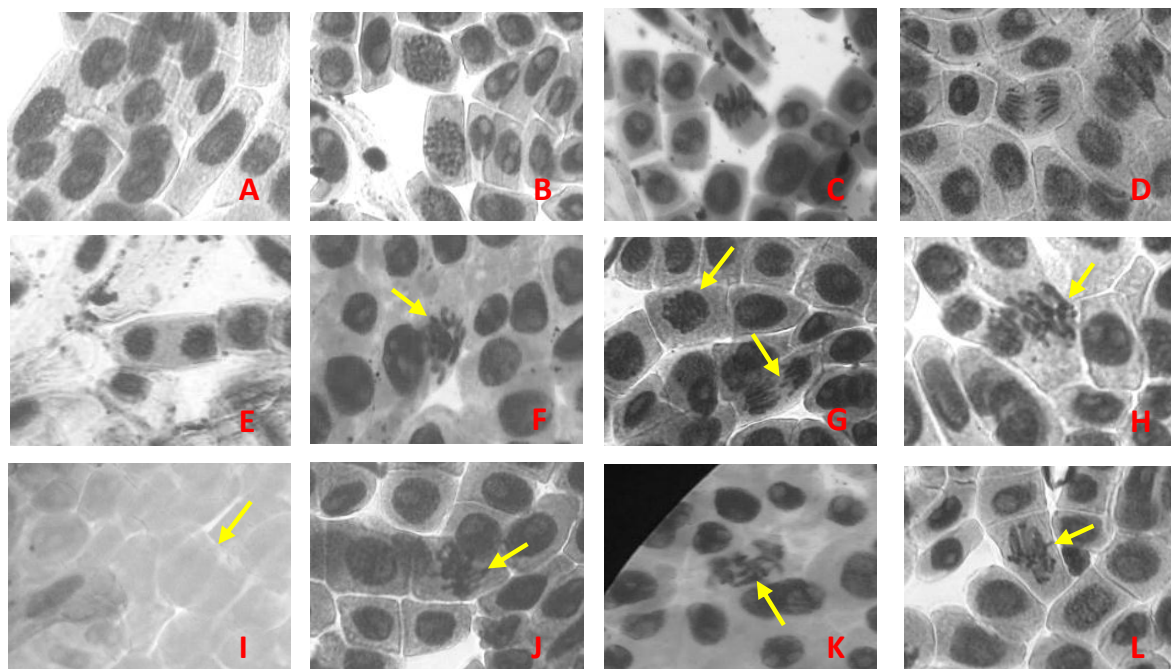
Treatment	Stickiness in Prophase	Stickiness in Metaphase	Disturbed Spindle	Chromosomal breaks	Disorganized chromosomes	MI of Non-aberrant Cells	MI of Aberrant Cells
MMS only	35	17	1	6	13	0.44 ± 0.23	1.73 ± 0.48
125 µg/mL BFE + MMS	12	18	28	6	15	2.02 ± 0.57	1.43 ± 0.42
250 µg/mL BFE+ MMS	19	18	19	3	14	2.07 ± 0.38	1.31 ± 0.35
500 µg/mL BFE+ MMS	9	22	15	3	13	2.36 ± 0.18	1.18 ± 0.27
1000 µg/mL BFE+ MMS	9	14	12	2	8	3.03 ± 0.14	0.86 ± 0.18
L-Ascorbic Acid + MMS	3	15	8	4	13	3.23 ± 0.37	0.83 ± 0.12
Deionized Water	1	2	0	0	0	3.26 ± 0.48	0.10 ± 0.10

\*A minimum of 5000 meristematic cells were assessed per treatment.



**Figure 4.** Antimutagenic Activity of Bignay Fruit Extract and L-Ascorbic Acid  
 \*Means with different letters are significantly different ( $p < 0.01$ )





**Figure 5.** Microscopic View of Onion (*Allium cepa L.*) Root Meristematic Cells  
 A = Interphase, B = Prophase, C = Metaphase, D = Anaphase, E = Telophase, F = Disturbed spindle, G = Sticky Prophase, chromosomal break, H = Sticky Metaphase, I = Ghost cells, J = Disturbed spindle, K = Disorganized chromosomes, L = Sticky Prophase

2008; Akeem, Mohamed, Asmawi, & Sofiman, 2011), however, the activity of bignay fruit to inhibit chromosomal aberrations due to DNA alkylation remains unexplored. The antioxidant properties of ripe bignay fruit extract in terms of radical scavenging activity was reported by Lizardo et al (2015) and El-Tantawy et al (2015). Similarly, this study reports the antioxidant capacity, lipid peroxidation inhibition, and antimutagenic activities of bignay fruit extract which is comparable to the activity of L-Ascorbic Acid, particularly at a concentration of 1000µg/mL. Our results suggest that bignay fruit extract has diverse antioxidant activities *in vitro*.

Based from the quantitative estimation of bioactive compounds, the fruit extract contained monomeric anthocyanins, flavonoids and phenolic compounds (Table 1), consistent with the reported data in the study of Amelia et al (2013), El-Tantawy et al (2015), and Lizardo et al (2015). Specific flavonoids such as catechin, procyanidin B1 and procyanidin B2 were already reported in the study of Butkhub & Samappito

(2008). Other compounds detected in ripe bignay fruits included epicatechin, rutin, reveratrol, quercetin, naringenin, kaempferol, luteolin, gallic acid, ferulic acid, and caffeic acid, although the presence of these bioactive compounds was dependent on the variety of the bignay berries (Bukthup & Samappito, 2011).

In our experiment, onion (*Allium cepa L.*) root meristematic cells were observed to be sensitive to the mutagenic effects of methyl methanesulfonate (Figure 5). The chromosomal aberration assay was performed by observing a minimum of 5000 cells per treatment to ensure the reliability of the results although a minimum of 1000 root meristematic cells is typically used to assess mutagenicity (Aşkin Celik & Aslantürk, 2010; Akeem et al., 2011). The *Allium cepa* assay has been used as a preferred *in vivo* test to assess both mutagenicity and antimutagenicity of plant extracts against several compounds through the evaluation of frequency and types of chromosomal aberrations (Tedesco & Laughinghouse IV, 2012). Generally, the mitotic indices (MI) in onion cells



treated with the bignay fruit extract + MMS were lower compared to the MI of cells exposed to L-Ascorbic Acid + MMS, but were considerably higher compared to the MI of cells treated with MMS only. There was a remarkable decrease in the occurrence of chromosomal aberrations in the root cells in a dose-dependent fashion when MMS was combined with bignay fruit extract, indicating potential antimutagenic activities of the extract against DNA alkylation as illustrated in Figure 4. A low mitotic index suggests a cytotoxic activity of the compound introduced to the root meristematic cells (Akeem et al., 2011), which means that MMS is cytotoxic.

It should be noted that bignay fruit extracts have been previously reported to exhibit toxic properties against *Artemia salina* (Micor, Deocaris, & Mojica, 2005) although exact mechanisms were not described. There is currently few data on the cytotoxicity of bignay. The observed reduction in the mitotic indices in onion root cells cannot be attributed to the fruit extract since they were mixed with MMS during the antimutagenicity assay. Furthermore, the fruit extracts were not genotoxic against DNA repair-deficient *E. coli* PQ37 in the SOS Chromotest genotoxicity assay. The strain *Escherichia coli* PQ37 [F<sup>-</sup> *thr leu his-4 pyrD thi galE galK or galT lacaU169 srl300::Tn10 rpoB rpsL uvrA rfa trp::Muc<sup>+</sup> sfiA::Mud(Ap,lac) ts*] has the *sulA::lacZ* fusion gene that indicates primary DNA damage induced during the SOS response (Quintero, Stashenko, & Fuentes, 2012). The SOS Chromotest assesses primary DNA damage and has been noted to predict potential mutagenicity aside from genotoxicity (Deng, West, Palu, & Jarakae Jensen, 2012). Since the concentration utilized in the genotoxicity assay did not result to genotoxicity in the bacterial cells, it is unlikely that bignay fruit extract may have caused the chromosomal aberrations observed in onion meristematic cells, in contrast to reports that some plant extracts can induce chromosomal aberrations to onion root cells (Aşkin Celik & Aslantürk, 2010). Furthermore, many antimutagenic agents may become co-mutagens after metabolic activation *in vivo* (Melo-Cavalcante, Picada, Rubensam, & Henriques, 2008).

In terms of specific cytogenetic effects (Table 3), most chromosomal aberrations observed in onion

root cells treated with the fruit extract + MMS and L-Ascorbic Acid + MMS were chromosomal stickiness particularly during metaphase, followed by disturbed spindles, disorganization of chromosomes and chromosomal breaks as illustrated in Figure 5. In root cells exposed to MMS only, the most common chromosomal aberration was stickiness during prophase (Table 3). This implies that both L-Ascorbic Acid and bignay fruit may have an ability to delay or reduce chromosomal aberrations. Very minimal chromosomal aberration was observed in the negative control, although this does not mean that deionized water induces chromosomal aberrations per se. The causes may be attributed to other factors not investigated in the study such as possible cellular stress during sample preparation, type of environmental conditions or quality of the onion samples.

MMS can cause direct methylation of the nucleophilic sites of the DNA, particularly on N-atoms, causing base modifications that often lead to DNA fragmentation due to the removal of alkali-labile abasic sites (Franke et al., 2005; Medeiros Mazzorana et al., 2013). Chromosomal stickiness is a consequence of an abnormal interaction of chromosomes that are attributed to error in protein-protein interaction or formation of complexes with the phosphate group of DNA (Nefic, Musanovic, Metovic, & Kurteshi, 2013) while disturbed spindles cause chromosomal breaks, disorganization of chromosomes and formation of ghost cells that are deficient in cytoplasmic structures; hence, these chromosomal aberrations may be related to each other (Aşkin Celik & Aslantürk, 2010).

Although exact mechanisms were not revealed in our study, it has already been theorized that phenolic compounds can be methylated by alkylating agents, protecting the DNA from alkylation (Franke et al., 2005). On the other hand, L-Ascorbic Acid was reported to block the covalent binding of alkylating agents to cellular DNA (Kaya, 2003). It is probable that bignay fruit also possesses dual antioxidant and antimutagenic properties against DNA alkylation due to the presence of phenolic compounds (Table 1) although other bioactive compounds need to be accounted for, as the extract is a complex mixture of several compounds which may exhibit varying activities. This study provides evidence

that bignay fruit extract may be a good source of compounds with antimutagenic properties against alkylating agents, aside from their robust antioxidant activities.

## Conclusion

Ripe bignay fruit is non-genotoxic and exhibits dose-dependent antimutagenic properties against DNA alkylation in onion root meristematic cells. At 1000 µg/mL, the highest antimutagenic property of the fruit extract was noted, similar to the activity of 1000 mg/mL of L-Ascorbic Acid. The highest antioxidant capacity and lipid peroxidation inhibition activity was also noted at 1000 µg/mL, although the lipid peroxidation inhibition activity was considerably lower compared to 1000 µg/mL of L-Ascorbic Acid. The fruit extract has a potential to be used as a source of antimutagenic compounds that do not exhibit genotoxicity.

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