

# FTIR Spectrum and Antimutagenicity of *Coffea arabica* Pulp and *Arachis hypogaea* Testa In Relation to their *In Vitro* Antioxidant Properties

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Jonathan M. Barcelo, Abigail M. Gatchallan, Iris Joy B. Aquino,  
Dhenize Rommenick E. Ollero, Francis Leonil D. Cortez,  
Trishia Mae C. Costales, Lloyd Allen Q. Marzo

Department of Medical Laboratory Science, School of Natural Sciences,  
Saint Louis University, Baguio City, Philippines  
jonathanbrcl@yahoo.com

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**Abstract** - Coffee pulp (*Coffea arabica*) and peanut testa (*Arachis hypogaea*) are common agricultural wastes which are extensively studied as potential sources of bioactive compounds. This study attempted to determine the correlation of the antioxidant potential, lipid peroxidation inhibition and antimutagenic activities of the crude extracts of both plant samples. Quantification of phenolic compounds, flavonoids and monomeric anthocyanins and Fourier Transform Infra-red (FTIR) analysis were performed to characterize bioactive compounds. Based from the results, peanut testa extract (PTE) exhibited higher total antioxidant capacity (TAC) compared to coffee pulp extract (CPE) at  $\leq 500 \mu\text{g/mL}$ . Higher lipid peroxidation inhibition (LPI) was noted in CPE at  $\geq 500 \mu\text{g/mL}$  but was lower than L-Ascorbic Acid (LAA). The antimutagenic activity of PTE at  $\geq 125 \mu\text{g/mL}$  was higher compared to both RPE and LAA. Mitotic indices of *A. cepa* treated with PTE+MMS were higher compared to groups treated with RCPE+MMS, LAA+MMS, deionized water and MMS only. Correlation analysis revealed the TAC of ripe coffee pulp extract had significant moderate correlation with its antimutagenic activity but high correlation with LPI. The LPI of CPE showed significant moderate correlation with its antimutagenic activity. In PTE, TAC shows high significant correlation with its antimutagenic property and LPI while LPI has significant moderate correlation with its antimutagenic property. The observed activity of the CPE and PTE may be attributed primarily to the phenolic compounds in both plant extracts.

**Keywords** – antimutagenicity, antioxidant capacity, *Arachis hypogaea* testa, *Coffea arabica* pulp, FTIR

## INTRODUCTION

Two of the most common agricultural waste products in the Philippines are coffee pulp and peanut testa. Peanut testa (seed coat or skin) is considered to be a low-value by-product of peanut blanching [1] while coffee pulp is considered to be one of the most abundant agricultural wastes [2] during depulping of coffee cherries. These two agricultural waste products are also generated in large quantities since peanut and coffee are major commercial agricultural crops in the Philippines.

Ripe coffee pulp and peanut testa are good sources of bioactive compounds and their antioxidant properties documented well. Coffee pulp was reported

to possess considerable antioxidant properties [3], [4]. Similarly, peanut testa is now recognized as a rich source of antioxidants [5], [6].

There are diverse studies on the antimutagenic potential of plant extracts against genotoxicants. Some studies reported that plant extracts which possessed antioxidant properties also exhibited antimutagenic properties against ROS-induced DNA damage. Interestingly, plant samples which are rich in phenolic compounds such as the seed coat of *Pisum sativum* [7] rhizomes of *Belamcanda chinensis* [8], and stems and leaves of *Anemopsis californica* [9] exhibited these two properties.

Some studies also investigated the potential of plants rich in phenolic compounds to inhibit the mutagenic effects of alkylating agents such as methylmethanesulfonate (MMS) *in vitro*. However, it seems that the presence of phenolic compounds in the plant extract does not necessarily predict the antimutagenic activity of plant extracts. For instance, the root extracts of *Plumbago zeylanica* has high phenolic content but exhibited poor antimutagenicity against MMS [10].

There is a limited data on the antimutagenic activity of ripe coffee pulp and peanut testa against alkylating agents although several studies have already reported their phenolic contents [3]–[6]. Hence, this study determined the effect of coffee pulp and peanut testa against methylmethanesulfonate (MMS) using *Allium cepa* chromosomal aberration assay. Total antioxidant capacity and lipid peroxidation inhibition activity were also performed to determine their possible relationship to antimutagenic activity against MMS. Phytochemicals such as phenolic compounds, flavonoids and anthocyanins were quantified and the functional groups were described using Fourier Transform Infra-Red (FTIR) Spectroscopy.

This study investigated the antimutagenic property and antioxidant activity of ripe coffee pulp extract and peanut testa extract as potential sources of phytochemicals. In addition, the correlation of the *in vitro* antioxidant activity and the antimutagenic activity against MMS was determined.

## MATERIALS AND METHODS

### Preparation of Samples

Three kilograms of ripe coffee (*Arabica* variety) and freshly harvested peanut (*Moket* variety) were obtained from Atok, Benguet, and Rosario, La Union, Philippines, respectively. The samples were washed with distilled water thrice to remove dirt. The ripe coffee pulps and peanut testa were manually separated, macerated and oven-dried at 80°C for 5 days. After drying, 500 grams of the samples was finely ground and extracted with 95% methanol for 72 hours at room temperature (22°C). The extracts were decanted, filtered twice with Whatman 1 Filter paper (0.45µm) and evaporated at 45°C. The residue was collected in an amber-coloured bottle and stored at -20°C until used.

### Determination of Total Phenolic Content

Total phenolic content was determined using the method described elsewhere [11]. Briefly, a 1mg/mL sample was mixed with 1mL of Folin-Ciocalteu reagent, then mixed with 5mL of 7% Na<sub>2</sub>CO<sub>3</sub> solution after 5 minutes and diluted with 13 mL of deionized water. The mixture was kept in the dark for 90 minutes. The absorbance of the mixture was determined at 750nm. A calibration curve using 0.01mg/mL to 2.00mg/mL of gallic acid standard was used to estimate the concentration of phenolic content in the plant extract. The total phenolic content was expressed as mg gallic acid equivalents (GAE) per 100g DW of extract. The procedure was performed in five replicates.

### Determination of Total Flavonoid Content

Total flavonoid content was determined by adding 0.3mL of 1mg/mL of the plant extract, 3.4mL 30% methanol, 0.15mL of 0.5M NaNO<sub>2</sub>, and 0.15mL of 0.3M AlCl<sub>3</sub>.6H<sub>2</sub>O based from literature [11]. After five minutes, 1mL of 1M NaOH solution was added. The absorbance was measured at 506nm against a reagent blank. Concentration was estimated using the calibration curve of quercetin dihydrate and is expressed as mg quercetin equivalents (QE) per 100g DW of extract.

### Determination of Total Anthocyanin Content

The total monomeric anthocyanin content of the sample was determined using pH differential method [12]. Two pH buffer solutions, Buffer 1 (pH 1.0, 1.86 g KCl in 1000 mL water) and Buffer 2 (pH 4.5, 54.43g of CH<sub>3</sub>COONa·3H<sub>2</sub>O in 1000mL water) were prepared and adjusted to the desired pH using concentrated HCl. In separate clean test tubes, 0.5 mL of the extract was mixed with 5 mL of pH 1.0 buffer solution and pH 4.5 buffer solutions. Buffer solutions were used as blank to adjust the absorbance of the diluted anthocyanin extracts. Spectrophotometric readings were measured at 520 nm and 700 nm according to the following equation:

$$\text{TAC} \left( \frac{\text{mg}}{\text{L}} \right) = \frac{A \times \text{MW} \times \text{DF} \times 1000}{\epsilon \times l} \quad (1)$$

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 100yaniding-3-glucoside equivalents (C-3-G eq, MW of C-3-G = 449.2 g·mol<sup>-1</sup>; DF = 10, l = 1 cm; ε = 26,900 L·cm<sup>-1</sup>·mol<sup>-1</sup>). The

procedure was performed in five replicates and the final unit was expressed as mg/100 g DW of the extract.

#### **Fourier Transform Infra-Red Analysis with ATR**

Fourier transform Infrared (FTIR) analysis was carried out in Shimadzu Laboratories, Taguig City, Philippines using Shimadzu IRTracer-100with PIKE ATR with ZnSe single bounce crystal [12]. Measurements were carried out at 20°C. The spectrum was recorded from 4500 to 750 cm<sup>-1</sup> by with a spectral resolution of 4 cm<sup>-1</sup>. An average of 30 scans was performed and % transmittance was recorded. The average peaks of the IR spectrum were analyzed using a reference literature [13].

#### **Determination of Total Antioxidant Capacity**

The phosphomolybdate method was used to estimate the total antioxidant capacity of the extracts [11]. In separate, clean test tubes, 0.1mL of the samples (125µg/mL to 1000µg/mL of the plant extract) 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 using paraffin film, incubated in a water bath at 95°C for 90 minutes then cooled to room temperature (22°C). The absorbance was measured at spectrophotometrically at 765nm against a reagent blank. The total antioxidant capacity was expressed as mg L-Ascorbic Acid equivalents.

#### **Inhibition of Lipid Peroxidation Hen Egg Yolk Homogenate**

In order to assess the inhibition of lipid peroxidation, thiobarbituric acid reacting species (TBARS) assay was performed using hen egg yolk homogenate according to two methods [14, 15]. In a clean test tube, 2mL of thiobarbituric acid reagent (20% glacial acetic acid and 0.67% 2-thiobarbituric acid in 0.25N HCl) was mixed with 0.5mL of buffered egg yolk homogenate and 0.5mL of the test solutions from 125µg/mL to 1000µg/assay.

To induce lipid peroxidation, 0.15mM FeSO<sub>4</sub>·7H<sub>2</sub>O was added, heated in a water bath for 30 minutes, cooled, and then centrifuged at 2000g for 5 minutes. The supernatant was collected and transferred to a glass cuvette and the absorbance was measured 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:

$$\% \text{ Inhibition} = \frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \times 100 \quad (2)$$

#### **Evaluation of Antimutagenicity of Extracts using Allium cepa Chromosomal Aberration Assay**

##### **Pre-treatment**

*Allium cepa* bulbs weighing about 15 to 20 grams were washed with distilled water and disinfected with 10% H<sub>2</sub>O<sub>2</sub> solution. The roots were removed to allow new root growth. The bulbs were grown in deionized water for 72 hours until new roots are about 2 – 3 cm long. The bulbs were transferred to the following treatments:

Positive Control:

Methyl methanesulfonate (MMS, 10µg/mL)

Experimental groups:

1000µg/mL coffee pulp extract + MMS

500 µg/mL coffee pulp extract + MMS

250 µg/mL coffee pulp extract + MMS

125 µg/mL coffee pulp extract + MMS

1000 µg/mL peanut testa extract + MMS

500 µg/mL peanut testa extract + MMS

250 µg/mL peanut testa extract + MMS

125 µg/mL peanut testa extract + MMS

Comparison group:

1000 µg/mL L-ascorbic acid + MMS

Negative control:

Deionized Water

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

##### **Slide Preparation**

Root cells harvested from 70% ethanol solution were prepared according to a method elsewhere [16]. The roots were rinsed with distilled water thrice and hydrolyzed in 1N HCl at 60°C to 70°C for 5 minutes. After hydrolysis, about 2 mm of the root tips were placed in a clean microscope slide. The root tip was stained with 2% orcein in 45% acetic acid solution. After two minutes, the root tip was squashed

with a blunt metal tip and stained again for two minutes. The cover slip was lowered carefully and sealed with a clear nail polish. The prepared slides were labelled appropriately and stored at 4°C until used. A total of 12 to 15 slides per treatment were prepared.

### Observation of Specimens

The slides were observed at 600x to 1000x magnification. Pictures of fields of view were taken using a digital camera (Canon Power Shot A490). A minimum of 1000 cells were evaluated per concentration per trial. Five trials were performed to obtain the mean ± standard deviation. The following data were obtained:

$$\text{Mitotic Index (MI)} = \frac{\text{Number of cells in mitosis}}{\text{Total Number of Cells}} * 100 \quad (3)$$

$$\%DR = \frac{[\text{MI AC (MMS)} - \text{MI AC (TG)}]}{\text{MI AC (MMS)} - \text{MI AC (NCG)}} * 100 \quad (4)$$

MI AC= mitotic index of aberrant cells  
 MMS = Methylmethanesulfonate  
 TG = Treatment Group  
 NCG = Negative Control Group

The % damage reduction reflects the antimutagenic activity of the plant extracts against methylmethanesulfonate (MMS).

### Statistical Tests

Data were presented using mean ± standard deviation, using five replicates. One way analysis of variance (ANOVA) with post hoc Tukey HSD test was utilized to determine the significant differences of the results based on concentration compared to the negative and positive controls. Statistical analysis was performed using IBM SPSS 20.0 at α=0.05. Correlation analysis was performed using Pearson Product Moment Coefficient of Correlation (r) at α=0.01.

## RESULTS AND DISCUSSION

### Total Phenolic, Flavonoid and Anthocyanin Content

Most of the extracted bioactive compounds are phenolic compounds. Peanut testa extract has higher total phenolic, total flavonoid and total monomeric anthocyanin content compared to ripe coffee pulp extract (Table 1).

### FTIR Spectrum

The FTIR spectrum revealed that coffee pulp and peanut testa may contain compounds such as alcohols,

phenolic compounds, alkenes, and carbonyl compounds (Table 2). A polymeric –OH stretching is a more prominent peak in the spectrum of ripe coffee pulp (3350.35 cm<sup>-1</sup>) while other peaks were observed to be similar in both samples.

### Total Antioxidant Capacity

Figure 1 shows that the total antioxidant capacity (TAC) is dose-dependent in both *C. arabica* pulp and *A. hypogaea* testa extract. The TAC of *A. hypogaea* testa extract is statistically higher compared to ripe coffee pulp extract at concentrations ≤ 500 µg/mL (p<0.05). However, the antioxidant capacity of the *C. arabica* pulp extract and *A. hypogaea* testa extract is comparable at 1000µg/mL.

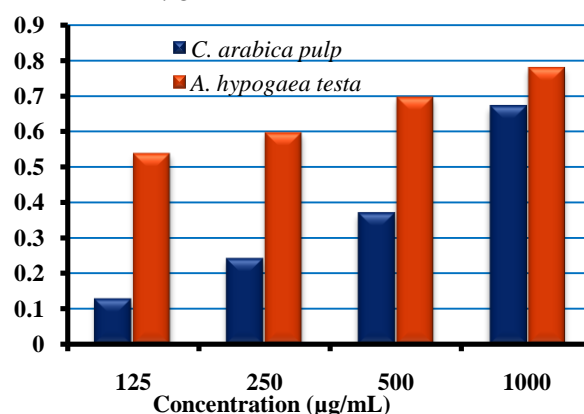


Fig. 1 Total Antioxidant Capacity *C. arabica* pulp and *A. hypogaea* testa expressed as L-Ascorbic Acid Equivalents

### Lipid Peroxidation Inhibition Activity

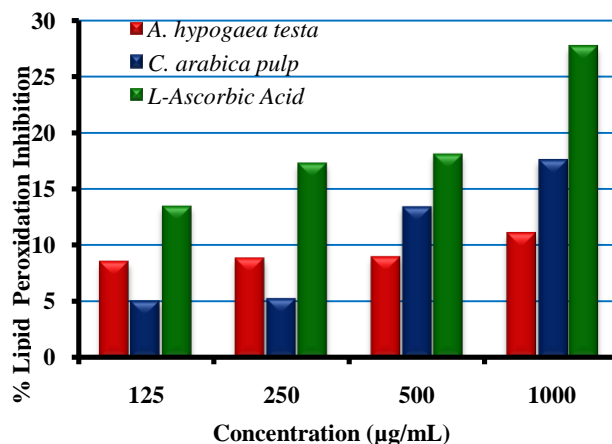


Fig. 2 Lipid Peroxidation Inhibition of *C. arabica* pulp and *A. hypogaea* testa compared to L-Ascorbic Acid

In Figure 2, the % lipid peroxidation inhibition is dose-dependent in L-Ascorbic Acid, *C. arabica* pulp and *A. hypogaea* testa. L-Ascorbic Acid has higher

lipid peroxidation inhibition activity compared to both plant extracts. At  $\geq 500 \mu\text{g/mL}$ , *C. arabica* pulp has greater ability to inhibit lipid peroxidation compared to *A. hypogaea* testa. It can be observed that the activity of *C. arabica* pulp extract is lower compared to *A. hypogaea* testa extract at  $\leq 250 \mu\text{g/mL}$  but displayed  $\sim 50.25\%$  and  $\sim 59.23\%$  higher lipid peroxidation inhibition compared to *A. hypogaea* testa extract at  $500 \mu\text{g/mL}$  and  $1000 \mu\text{g/mL}$ , respectively.

**Effects of *C. arabica* pulp and *A. hypogaea* testa on *A. cepa* root cells treated with MMS**

Based from the % damage reduction shown in Table 4, the crude extract of peanut testa possesses significantly higher antimutagenic activity against MMS compared to L-Ascorbic Acid and coffee pulp extract. However, the antimutagenic activity of coffee pulp extract is statistically comparable to L-Ascorbic Acid ( $\rho > 0.05$ ). Furthermore, coffee pulp extract poorly reduced the mitodepressive effect of MMS compared to L-Ascorbic Acid and peanut testa extract but moderately reduced the chromosomal aberrations caused by MMS.

**Correlation of Antioxidant and Antimutagenic Properties of *C. arabica* pulp and *A. hypogaea* testa extract**

Table 3 shows that the antimutagenic activity of *C. arabica* extract and *A. hypogaea* testa extract is concentration-dependent ( $\rho < 0.01$ ). However, mitotic indices revealed very low negative correlation to the concentration of the extracts ( $\rho > 0.01$ ).

In *C. arabica* pulp extract, total the antioxidant capacity is moderately correlated to antimutagenic property but has very high correlation to inhibition of lipid peroxidation activity. The lipid peroxidation inhibition activity of the extract is also moderately correlated to its antimutagenic activity.

The crude extract of *A. hypogaea* also revealed that its antimutagenic activity against MMS in *Allium cepa* cells is highly correlated to total antioxidant capacity but is moderately correlated to lipid peroxidation inhibition ( $\rho < 0.01$ ). Mitotic index, on the

other hand, shows insignificant low correlation to total antioxidant capacity and lipid peroxidation inhibition ( $\rho > 0.01$ ).

**DISCUSSIONS**

The antimutagenic properties of plant extracts have been reported to show relationship with their *in vitro* antioxidant activities [7, 8]. When cellular damage was induced by reactive oxygen species (ROS) such as hydroxyl radicals, superoxide radicals and  $\text{H}_2\text{O}_2$ , the antioxidant mechanisms of plant extracts contribute to genotoxicity modulation or suppression. However, it is not yet clear if the antioxidant properties of plant extracts also play a role in inhibiting or suppressing the mutagenic property of alkylating agents such as methylmethanesulfomate (MMS).

One study [17] presented a high phenolic content, high antioxidant activity but moderate genotoxicity inhibition of *Codium tomentosum* ethanolic crude extract against ethylmethanesulfonate (EMS) in human lymphocytes. Using the *Allium cepa* chromosomal aberration assay, the crude extracts of *Clinacanthus nutans*, *Adhatoda vasica*, and *Carica papaya* were reported to possess antimutagenic properties against methylmethanesulfonate [18]. These data suggest that plant extracts not only possess antigenotoxic activities against oxidative damage; they may also contribute to antimutagenic activities against alkylating agents.

Studies involving the antimutagenic activity of ripe coffee pulp and peanut testa against alkylating agents are uncommon. Ripe coffee pulp and peanut testa are common agricultural wastes in the Philippines although these plant samples contain phenolic compounds [3, 6]. Hence, this study focused on determining the relationship of the antimutagenic activity of these samples in relation to their *in vitro* antioxidant capacity and lipid peroxidation inhibition activity. This study quantified phenolic compounds, flavonoids and monomeric anthocyanins using colorimetric assays.

Table 1. Phenolic, Flavonoid and Anthocyanin Content of *Coffea arabica* Pulp and *Arachis hypogaea* Testa Extracts

Bioactive Compounds	Concentration (mg/100g DW of extract)	
	<i>C. arabica</i> Pulp Extract	<i>A. hypogaea</i> Testa Extract
Total Phenolic Content	439.64 ± 32.73 GAE	9560.36 ± 313.02 GAE
Total Flavonoid Content	46.22 ± 3.48 QE	820.89 ± 5.85 QE
Total Anthocyanin Content	60.95 ± 4.82 C3GE	172.83 ± 18.97 C3GE

Table 2. Average FTIR peaks of *Coffea arabica* pulp and *Arachis hypogaea* testa

<i>Coffea arabica</i> Pulp		<i>Arachis hypogaea</i> Testa	
Peak (cm <sup>-1</sup> )	Functional Group	Peak (cm <sup>-1</sup> )	Functional Group
719.45	Alcohol, -OH out of plane bend	721.38	Aromatic C – H out of plane bend
1043.49	Cyclohexane ring vibrations	1051.20	Cyclohexane ring vibration
1238.30	Aromatic ethers, aryl – O stretching	1176.58	Tertiary amine, C – N stretch
1377.17	Carboxylate group	1280.73	Primary or secondary alcohol, in-plane bend
1458.26	Methyl C – H asymmetric/ symmetric bending	1323.17	Phenol or tertiary alcohol, OH in plane bend
1653.00	Alkenyl C=C stretch	1377.17	Phenol or tertiary alcohol, OH in plane bend
1707.00	Carbonyl group	1411.89	Vinyl C – H in-plane bend
1732.08	Carbonyl group	1463.97	Aromatic ring stretch
2852.72	Methylene C – H asymmetric/ symmetric stretch	1622.13	Stretching of alkenyl group (C = C)
2922.16	Methylene C – H asymmetric/ symmetric stretch	1708.93	Carbonyl group
3350.35	-OH stretching	1739.79	Carbonyl group
		2852.72	Methylene C – H asymmetric/ symmetric stretch
		2922.16	Methylene C – H asymmetric/ symmetric stretch

Table 3. Correlation of Variables

	<i>Coffea arabica</i> Pulp				<i>Arachis hypogaea</i> Testa			
	MI	AMA	TAC	LPI	MI	AMA	TAC	LPI
Concentration	-0.283	0.628*	0.997*	0.940*	0.253	0.853*	0.967*	0.896*
TAC	-0.265	0.657*	-	0.924*	0.194	0.937*	-	0.804*
ALP	-0.271	0.568*	-	-	0.186	0.670*	-	-

\*significant at  $\alpha=0.01$ ; MI = Mitotic Index; AMA= Antimutagenic Activity; TAC = Total Antioxidant Capacity; LPI = Lipid Peroxidation Inhibition

Table 4. Cytogenetic Effects of *Coffea arabica* Pulp Extract, *Arachis hypogaea* Testa Extract and L-Ascorbic Acid against Methylmethanesulfonate in *Allium cepa* meristematic root cells

Compounds Tested	Concentration (µg/mL)	Total Number of Cells	Stickiness (Metaphase)	Bridges	Nuclear fragmentation	Stickiness (Prophase)	Disturbed Spindle	Average MI	MI <sub>NC</sub>	MI <sub>AC</sub>	DR (%)
<b>MMS</b>	10	5393*	38	7	7	0	9	1.99 ± 0.80 <sup>e</sup>	0.43 ± 0.22	1.69 ± 0.47	-
<b>LAA + MMS</b>	1000	5514	18	4	6	2	9	4.07 ± 0.36 <sup>d</sup>	3.16 ± 0.37	0.87 ± 0.17	53.54 ± 10.87 <sup>b</sup>
<b>CPE + MMS</b>	1000	5584*	29	4	6	6	8	2.87 ± 0.49 <sup>e</sup>	1.96 ± 0.45	0.98 ± 0.17	41.10 ± 17.42 <sup>b</sup>
	500	5701*	34	9	8	8	2	2.98 ± 0.44 <sup>e</sup>	1.91 ± 0.40	1.10 ± 0.13	33.62 ± 15.16 <sup>b</sup>
	250	5600	38	13	2	14	0	3.15 ± 0.65 <sup>b</sup>	1.95 ± 0.44	1.24 ± 0.34	28.12 ± 5.97 <sup>c</sup>
	125	5744	33	16	8	16	0	3.27 ± 0.60 <sup>b</sup>	1.93 ± 0.49	1.54 ± 0.45	9.08 ± 6.02 <sup>c</sup>
<b>PTE + MMS</b>	1000	5768*	7	5	0	0	3	5.20 ± 0.19 <sup>a</sup>	4.59 ± 0.13	0.37 ± 0.09	81.70 ± 5.00 <sup>a</sup>
	500	5906*	9	9	10	0	5	4.71 ± 0.63 <sup>a</sup>	4.15 ± 0.54	0.59 ± 0.31	68.56 ± 17.29 <sup>b</sup>
	250	5984	22	19	8	8	5	4.78 ± 0.83 <sup>a</sup>	3.74 ± 1.02	1.09 ± 0.27	36.70 ± 7.05 <sup>b</sup>
	125	5794	37	18	6	12	12	4.88 ± 0.51 <sup>a</sup>	3.41 ± 0.56	1.47 ± 0.45	14.51 ± 6.69 <sup>c</sup>
<b>DW</b>	-	5703	3	0	0	0	1	3.26 ± 0.56 <sup>b</sup>	3.07 ± 0.47	0.09 ± 0.09	-

Means with different letter superscripts are significantly different ( $p \leq 0.05$ )

MI<sub>NC</sub> = mitotic index of normal cells

MI<sub>AC</sub> = mitotic index of aberrant cells

\*Ghost cells were observed

CPE = Coffee Pulp Extract

PTE = Peanut Testa Extract

LAA = L-Ascorbic Acid

DW = Deionized Water

MMS = Methylmethanesulfonate

Furthermore, the functional groups were revealed using FTIR analysis to characterize common functional groups which may play a role in the observed activities of the crude extracts. Based from the FTIR spectra, the average peaks revealed the presence of alcohol and phenolic functional groups in both extracts, indicating the presence of phenolic compounds. Peanut testa and ripe coffee pulp extracts were also revealed to possess carbonyl functional groups associated with carboxylic acids. This result can be substantiated by other studies since *p*-coumaric acid, ferulic acid, caffeic acid and chlorogenic acid were commonly detected in coffee pulp extracts [4] while *p*-hydroxybenzoic acid, *p*-coumaric acid and ferulic acid are detected in peanut testa (Win *et al.* 2011).

Phenolic compounds are associated with the antioxidant activity of crude extracts [17, 19] although other compounds in the crude extract may modulate the net antioxidant activity. Methanolic fractions of peanut testa was reported to possess appreciable antioxidant activities particularly in the methanolic fraction, although combined methanolic and ethyl acetate fractions may also exhibit comparable DPP• inactivation, HO• inactivation and superoxide inactivation [20] while aqueous methanolic crude fractions of coffee pulps has been recognized to exhibit antioxidant activities due to their hydroxycinnamic acid content [4]. Crude extracts were used in this study because pure, isolated compounds may possess greater toxicity [21].

Lipid peroxidation is the degradation of unsaturated fatty acids caused by free radicals leading to membrane damage and injury to proteins and DNA [22, 23]. The nature of the fatty acid content and the presence of saturated versus unsaturated fatty acids, determines the degree of inhibition of thiobarbituric acid reactive species (TBARS) production, which is a hallmark of peroxidation [22]. Hence, a possible mechanism to prevent oxidative damage to DNA may be assessed by the inhibition of lipid peroxidation. Lipid peroxidation may be caused by autooxidation through free radical initiation, photooxidation and enzymatic action on unsaturated and saturated fatty acids [24]. Lipid peroxidation in hen egg yolk homogenate was induced by adding FeSO<sub>4</sub>. Hence, the inhibition caused by peanut testa extract and coffee pulp extract is may be related to their antioxidant capacities.

Egg yolk homogenate is a complex mixture of lipids and other compounds which could possibly react with some of the bioactive compounds in the extracts. The results imply that egg yolk homogenate is sufficient in comparing the lipid peroxidation inhibition of the crude plant extracts and L-Ascorbic Acid. It has been suggested that phenolic compounds efficiently inhibit lipid peroxidation in egg yolk homogenate [15]. Phenolic compounds may act as chelator of ions, preventing hydroxyl radicals from participating in oxidative damage that promotes lipid peroxidation [25]. Contrary to its antioxidant capacity, peanut testa has lower lipid peroxidation inhibition activity compared to coffee pulp extract despite having a high phenolic content (Table 1). Perhaps, other compounds in peanut testa exhibit antagonistic effects to the activity of phenolic compounds *in vitro* systems.

Crude plant extracts may be genotoxic or antigenotoxic, clastogenic or cytotoxic [21] but their net effect is still dependent on the activity of the interaction of compounds present in the extract and the concentration used in performing the assay. The *Allium cepa* chromosomal aberration assay is widely used to test the cytotoxicity and genotoxicity of plant extracts. The antigenotoxic and antimutagenic properties of plant extracts against toxicants were also assessed using variations of this assay [26, 27]. In our study, methylmethanesulfonate (MMS) was used to induce chromosomal aberrations to meristematic cells of *Allium cepa*. Based from previous reports, MMS induces DNA damage by causing alkyl additions to nitrogenous bases of DNA which leads to alteration of base pairing [27, 28].

The mechanism of antimutagenicity of plant extracts is generally complex [29]. Crude extracts may act as non-specific redox agents, free radical scavengers, and ligands to toxicants [21]. Our results suggest that the antioxidant activity of the extracts is correlated to their antimutagenic activity in *Allium cepa* chromosomal aberration assay. Hence, antioxidative mechanisms may play a role on the ability of coffee pulp and peanut testa to inhibit the mutagenic effects of MMS. The compounds in the crude extracts of coffee pulp and peanut seed coat may have combined effects against the mutagenic effect of MMS. Intracellularly, the antimutagenic mechanisms of plant extracts proposed in one study [7] include union to DNA, proteins and lipids, protection of the nucleophilic sites of DNA,

modification of DNA repair, enhancement of DNA replication fidelity or inhibition of prone-error DNA system repair. L-Ascorbic Acid was suggested to block MMS in forming covalent bonding with DNA, thereby modulating the mutagenicity of the alkylating agent [30]. Although the data presented in this study do not fully explain how crude extracts inhibit the clastogenicity of MMS, earlier studies have suggested that crude plant extracts may act as desmutagenic agents that are capable of chemically inactivating MMS when they are mixed together before the incubation of *Allium cepa* root cells [26, 27]. It is probable that both crude extracts of *C. arabica* pulp and *A. hypogaea* testa may promote antimutagenic properties using both intracellular and extracellular mechanisms.

This study adds to the body of evidence that the antioxidant capacity and inhibition of peroxidation activity of crude plant extracts may be related to the inhibition of the mutagenic effects of MMS, an alkylating agent. However, we also recognize that a single assay is not sufficient to conclude that the crude extracts of ripe coffee pulp and peanut testa possess anti-cancer properties. It is highly likely that the extracts of ripe coffee pulp and peanut testa may contribute to the reduction of oxidative and non-oxidative DNA damage in cells *in vitro* using non-specific mechanisms. It is not yet clear in this study if the bioactive compounds in the crude plant extracts of *C. arabica* pulp extract and *A. hypogaea* testa extracts are synergistic or antagonistic in exhibiting their antioxidant capacity, lipid peroxidation inhibition, and antimutagenic activity.

The report on the FTIR spectrum, antioxidant capacity, lipid peroxidation inhibition and antimutagenic property of the crude extracts of ripe coffee pulp and peanut testa against MMS can be used as a basis for further investigations on the utilization of these agricultural wastes as sources of phytochemicals.

#### CONCLUSION AND RECOMMENDATION

The *in vitro* antioxidant properties of ripe coffee pulp and peanut testa are correlated to their antimutagenic activity against MMS using the *A. cepa* chromosomal aberration assay. Peanut testa possesses higher antioxidant capacity *in vitro* and higher antimutagenic activity compared to ripe coffee pulp. At higher concentrations, coffee pulp has higher lipid peroxidation inhibition compared to peanut testa. These

activities might be attributed to the phenolic compounds and other bioactive compounds which bear the functional groups revealed by their FTIR spectra.

It is recommended that the solvent fractions of the extracts should be investigated using other mutagenic agents *in vitro* and *in vivo*, since this was not performed in the study. In addition, the synergistic effects of the extracts can be further investigated. Since the mechanism of the antimutagenic activity was not elucidated, it is further recommended that other assays should be performed using animal models.

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