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## Antioxidant Potential of Coconut Flour in Caco-2 Colon Cancer Cells

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### ABSTRACT

Coconut Flour (CF) (*Cocos nucifera* L.) is a sweet by-product of coconut milk that has multifarious utility. The objective of this study was to investigate the chemopreventive potential of coconut flour extracts (defatted and whole) through determination of phytochemical content, antioxidative potential and cytotoxic effects on Caco-2 cells. Phytochemical (total phenolic, total flavonoid) content, Trolox Equivalent Antioxidant Capacity (TEAC), free radical-scavenging ability of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric Reducing Antioxidant Potential (FRAP) were determined according to standard protocols. Lactate dehydrogenase (LDH) release (cytotoxicity assay) was used to measure *in vitro* cell damage of Caco-2 colon cancer cells. Activity of antioxidant enzymes was determined with selected concentrations of extracts (25, 50 and 100  $\mu\text{g mL}^{-1}$ ). Apoptotic activity was assessed through the activity of caspase-3 and DNA fragmentation Enzyme-Linked Immunosorbant Assay (ELISA). Anti-inflammatory activity was assessed through measuring cyclooxygenase-2 (Cox-2). Total phenolic and flavonoid content was  $55.46 \pm 1.70$  mg GAE/100 g and  $17.84 \pm 2.33$  mg CE/100 g. The TEAC was  $0.65 \pm 0.05$   $\mu\text{mol TE}/100$  g. The DPPH was  $\text{IC}_{50}$ -74 mg  $\text{mL}^{-1}$ . The FRAP was  $0.31 \pm 0.21$  mmol/ $\text{Fe}^{+2}$   $\text{g}^{-1}$  and ORAC was  $2387.78 \pm 88.68$   $\mu\text{M TE g}^{-1}$   $14.13 \pm 1.25$  mmol/ $\text{Fe}^{+2}$   $\text{g}^{-1}$ . The LDH (%) release for 12 and 24 h was 45.8% at 50  $\mu\text{g mL}^{-1}$  and 63.5% at 25  $\mu\text{g mL}^{-1}$ . Caspase-3 was  $15.46 \pm 0.639$ , Cox-2 was 0.30 U  $\text{mL}^{-1}$  and DNA fragmentation ELISA was 0.39 EF at 50  $\mu\text{g mL}^{-1}$ . The results for the antioxidant and detoxification enzymes yielded: Glutathione-S-Transferase (GST)  $2.12 \pm 0.32$  nmol  $\text{min}^{-1}$   $\text{mL}^{-1}$ , catalase (CAT)  $24.65 \pm 0.40$  U  $\text{mg}^{-1}$ , Glutathione Peroxidase (GPx)  $30.4 \pm 0$  nmol  $\text{min}^{-1}$   $\text{mL}^{-1}$ , superoxide dismutase (SOD)  $305.90 \pm 3.89$  U  $\text{mL}^{-1}$  and glutathione (GSH)  $24.4 \pm 0$  nmol  $\text{mL}^{-1}$ . Results of this study revealed high phenolic and flavonoid content and suggested cytotoxic and antioxidative potential of coconut flour extracts.

**Key words:** Phytochemicals, inhibition, chemoprevention, colorectal cancer, antioxidants, coconut flour

### INTRODUCTION

Cancer is a growing problem that can affect various organs once it enters the lymphatic system. Interestingly, the cancer does not always originate in the organ that it has metastasized to. Colorectal cancer is cancer that originates in the digestive tract and subsequently metastasizes in

the colon or rectum. Cancer can be the outcome of various factors including oxidative stress, diet and genetic factors. A genetic predisposition for mendelian tumor syndrome can increase an individual's risk of developing colorectal adenomas (Lamlum *et al.*, 2000). Overexpression of enzymes like cyclooxygenase-2 (Cox-2) and chromosomal abnormalities have all been linked to cause adenoma formation (Gustin and Brenner, 2002). Diet is often the best preventive option because it is a major cause attributed to the link of colorectal cancer (WCRFI., 2011; Biasi *et al.*, 2013; Giovannucci *et al.*, 1995; Oba *et al.*, 2006; Gustin and Brenner, 2002; Russo, 2007).

*Cocos nucifera* L. (coconut), grown in tropical and subtropical regions and is an excellent source of energy, dietary fiber and vitamin B (thiamin, niacin and riboflavin etc.) (Yong *et al.*, 2009). A staple product in Asian cuisine, coconuts have multifarious uses (sugar, milk and water) and is ideal for utilization within the food industry. Coconut, coconut by products, such as water and milk, contain phytohormones like cytokinins that function in the growth of plants (Kieber and Schaller, 2014). Coconuts are reported to decrease the loss of trabecular bone thickness and number (Hayatullina *et al.*, 2012). The presence of phytochemicals such as phenolic acids, flavonoids, tannins and epicatechins can act as antioxidants by suppressing cancer-causing agents. In coconuts, these phytochemicals can have anti-inflammatory function in the prevention of rheumatoid arthritis and antioxidant function in the down-regulation of Cox-2 (Vysakh *et al.*, 2014; Chang and Wu, 2011). In the food industry there are promising uses of coconut flour. Trinidad *et al.* (2006) reported the utilization of coconut flour as a source of dietary fiber in various snack products which significantly reduce serum and Low Density Lipoprotein (LDL) cholesterol (6.9 and 10.8%) in normal and type II diabetes mellitus *in vivo* test subjects. *In vivo* studies on rat models have shown the osteo-preventive effects of coconut (Radenahmad *et al.*, 2006; Yusuh *et al.*, 2010). Presently, there have been no studies on the antioxidant and chemopreventive effects of coconut flour. Thus, this study focused on determining the *in vitro* effects of Coconut Flour (CF) extracts on detoxification and anti-oxidative enzymes in addition to assessing the anti-inflammatory, antioxidant and cytotoxicity effects of CF.

## **MATERIAL AND METHODS**

**Sample preparation:** Chemicals of pure analytical grade were obtained from Sigma Chemical Company, (St Louis, MO.) and Fisher Scientific Company (Suwanne, GA). Raw Coconut Flour (CF) (*Cocos nucifera* L.) was obtained from a local health food store, Huntsville, Alabama, US. The CF samples were defatted utilizing the soxhlet apparatus distillation flask. The samples were refluxed with n-hexane (200 mL) and dried overnight at 25°C. For whole extractions CF mixture was extracted by stirring for 2 h at 25 and 170°C. The samples were filtered before being evaporated to dryness at 40°C using a rotary evaporator (Safe-Aire, Fisher Hamilton, Gaithersburg, MD, US). The CF extract samples were stored at -20°C until further analysis (Weisz *et al.*, 2009; Seneviratne *et al.*, 2009).

**Determination of total phenolic and flavonoid content:** Total phenolic content was determined through the use of the Folin Ciocalteu reagent, as outlined by Kim *et al.* (2002) and expressed as milligram Gallic Acid Equivalent (GAE)/100 g dry weight. Total flavonoid content was determined by colorimetric assay as described by Zhishen *et al.* (1999) with modifications and expressed as Catechin Equivalents milligram (CE)/100 g dry weight.

**2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability:** The DPPH radical scavenging activity was determined using the methods of Kim *et al.* (2002) and Lee *et al.* (2003). The free radical DPPH at a concentration of 0.1 mM was added to the sample mixture. Percentage inhibition of DPPH was determined using the following calculation:

$$\text{Inhibition of DPPH (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \quad (1)$$

**Ferric Reducing Antioxidant Power (FRAP):** Ferric Reducing Antioxidant Power (FRAP) was based on the method outlined by Benzie and Strain (1999) with modifications and expressed as mmol Fe<sup>2+</sup> g<sup>-1</sup> dry weight.

**Trolox Equivalent Antioxidant Capacity (TEAC):** Trolox Equivalent Antioxidant Capacity (TEAC) was measured utilizing modified method of Zulueta *et al.* (2009) and results were expressed as μmol TE/100 g.

**Oxygen-Radical Absorbance Capacity (ORAC):** Oxygen-Radical Absorbance Capacity (ORAC) assay was based on the method by Zulueta *et al.* (2009) with modifications. The AAPH (2,2-azino-bis(2-amidinopropane) dichloride) was used to generate the radical species.

The following formula was used to calculate the ORAC activity:

$$\text{AUC} = 1 + \text{RFU}_1/\text{RFU}_0 + \text{RFU}_2/\text{RFU}_0 + \text{RFU}_3/\text{RFU}_0 + \dots + \text{RFU}_{60}/\text{RFU}_0 \quad (2)$$

where, AUC is the area under the curve and RFU<sub>0</sub> is the relative fluorescence value at time zero.

**Cell culture:** Human colon cancer cells Caco-2 cell (American Type Culture Collection (ATCC, Manassas, VA)) was maintained in Dulbecco's Modified Eagles Media (DMEM) with 10% Fetal Bovine Serum (FBS) and seeded in a 24 well culture plate and incubated at 37°C and 7% CO<sub>2</sub> until the development of a monolayer for assay.

**Lactate Dehydrogenase (LDH) assay:** Cells were incubated for 12 and 24 h in CF extracts (25-400 μg mL<sup>-1</sup>) prepared with fresh media (DMEM serum free). Cellular cytotoxicity was measured by lactate dehydrogenase (LDH) release *in vitro* by cell damage of Caco-2.

**Enzyme assays:** The activities of all enzymes and Glutathione-S-Transferase (GST) activity, Glutathione Peroxidase (GPx) activity, Superoxide dismutase (SOD) activity, Catalase (CAT) activity and reduced glutathione (GSH) were determined spectrophotometrically at various wavelengths using the methods outlined in the Cayman's Assay Kits (Cayman Chemical Company, Ann Arbor, MI, US) (Griffith, 1985; Habig *et al.*, 1974; Jaskot *et al.*, 1983; Johansson and Borg, 1988).

**Cyclooxygenase (Cox-2):** Cyclooxygenase (Cox-2) activity (nmol min<sup>-1</sup> mL<sup>-1</sup>) measured the oxidation of tetramethyl-p-phenylenediamine (TMPD) at 590 nm for a total of for a total of 5 min at 25°C (Kulmacz and Lands, 1983).

**Apoptotic activity:** Apoptosis in human colon cancer cells (Caco-2) was detected measuring caspase-3 activity colorimetrically (405 nm) and cellular DNA fragmentation Enzyme-Linked Immunosorbant Assay (ELISA) spectrophotometrically (450 and 690 nm) according to manufactured kit instructions.

**Statistical analysis:** Data obtained from chemical analysis is reported as Mean±SEM and analyzed using the SAS 9.1. Significant differences among treatment groups and means were determined using ANOVA and Tukey's standardized range test. The significance level was established at  $p \leq 0.05$ .

## RESULTS

**Total phenolic and total flavonoid content:** The Total Phenolic and Total Flavonoid Content (TPC and TFC) of Coconut Flour (CF) extracts is shown in Table 1. The CF extracts TPC ranged from 30.36±1.07 to 55.46±1.70 mg GAE/100 g. Highest total phenolic contents of coconut flour (mg GAE/100 g) was observed in defatted -25°C, which was significantly ( $p < 0.05$ ) higher than whole (25 and 170°C) and defatted -170°C. A significant ( $p < 0.05$ ) decrease was observed in defatted -170°C (30.36 mg GAE/100 g). Total flavonoid content (CE/100 g) of CF showed a significant ( $p < 0.05$ ) increase in defatted -25°C (17.84 mg GAE/100 g) and no significant differences were observed between the remaining extraction methods whole -25°C, whole -170°C and defatted -170°C coconut flour.

**DPPH scavenging activity:** The free radical (DPPH) scavenging activity of CF extracts exhibited a concentration dependence (Fig. 1) in the inhibition of DPPH radical, which, ranged from 2-60%. Highest free radical (DPPH) scavenging activity of CF extracts was at defatted -170°C ( $IC_{50}$ -62 mg mL<sup>-1</sup>).

Highest ABTS scavenging ability (Table 2) was at 0.65 µmol TE/100 g in defatted -25°C. Antioxidant activity was observed among extractions whole -25°C (0.53 µmol TE/100 g), whole -170°C (0.61 µmol TE/100 g) and defatted -170°C (0.58 µmol TE/100 g) and defatted -25°C (0.65 µmol TE/100 g). Ferric reducing antioxidant power (Table 2) of CF extracts did not show significant differences among extractions, however, highest FRAP value was in whole and defatted -25°C (0.31 mmol/Fe<sup>+2</sup> g<sup>-1</sup>). Oxygen-Radical Absorbance Capacity (ORAC) assay (Table 2) Whole -170°C CF was significantly ( $p < 0.05$ ) higher among extraction methods and no significant difference was reported between whole -25°C and defatted -25°C.

Table 1: Total phenolic and flavonoid content of coconut flour extracts

Coconut flour	Total phenolic content (mg GAE/100 g)	Total flavonoids content (mg CE/100 g)
Whole -25°C	43.06±0.17 <sup>b</sup>	6.18±0.15 <sup>b</sup>
Whole -170°C	40.86±1.37 <sup>b</sup>	7.13±0.49 <sup>b</sup>
Defatted -25°C	55.46±1.70 <sup>a</sup>	17.84±2.33 <sup>a</sup>
Defatted -170°C	30.36±1.07 <sup>c</sup>	5.78±0.31 <sup>b</sup>

Values are Means±SEM, n = 3. <sup>abc</sup>Values not sharing common superscript are significantly different ( $p < 0.05$ ) by Tukey's studentized range test. Whole and Defatted: Extraction at temperatures of 25 and 170°C, GAE: Gallic acid equivalents, CE: Catechin equivalents

Table 2: Antioxidant potential of coconut flour extracts

Coconut flour	TEAC (µmol TE/100 g)	FRAP (mmol/Fe <sup>+2</sup> g <sup>-1</sup> )	ORAC (µM TE g <sup>-1</sup> )
Whole -25°C	0.53±0.04 <sup>a</sup>	0.31±0.20 <sup>a</sup>	163.24±3.94 <sup>c</sup>
Whole -170°C	0.61±0.03 <sup>a</sup>	0.23±0.14 <sup>a</sup>	2387.78±21.68 <sup>a</sup>
Defatted -25°C	0.65±0.05 <sup>a</sup>	0.31±0.21 <sup>a</sup>	141.56±2.03 <sup>c</sup>
Defatted -170°C	0.58±0.03 <sup>a</sup>	0.06±0.03 <sup>a</sup>	1324.93±18.43 <sup>b</sup>

<sup>abc</sup>Values are Means±SEM, (n = 3) not sharing common superscript are significantly different ( $p < 0.05$ ) by Tukey's studentized range test. Whole and defatted: Extraction at temperatures of 25 and 170°C, FRAP: Ferric reducing antioxidant power, Fe<sup>2+</sup>: Ferrous ion, ORAC: Oxygen-radical absorbance capacity assay and TEAC: Trolox equivalent antioxidant capacity

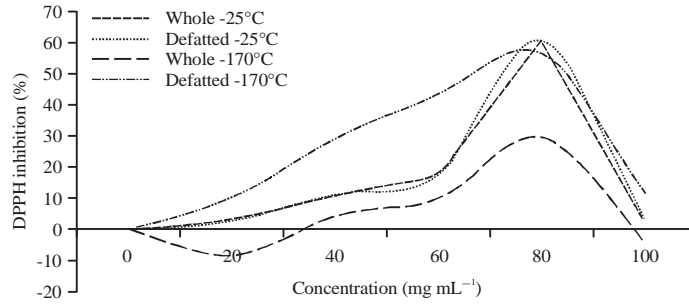


Fig. 1: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of Coconut Flour (CF) extracts

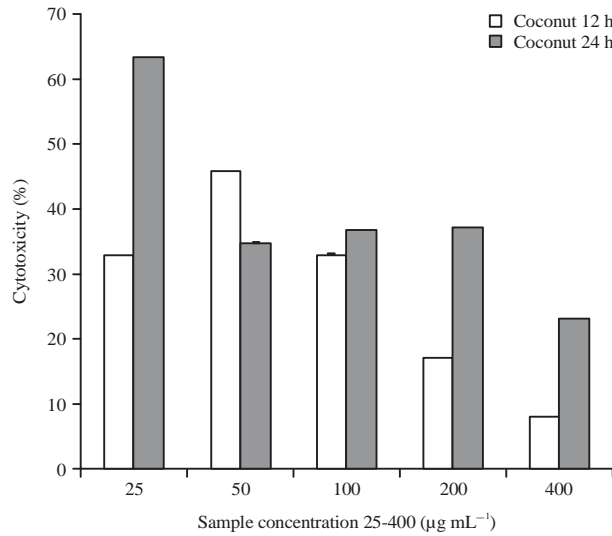


Fig. 2: Lactate dehydrogenase release (cytotoxicity) of Caco-2 cells treated with Coconut Flour (CF) extracts for 12 and 24 h

### Cell culture

**Lactate dehydrogenase (cytotoxicity (%)):** The LDH percent cytotoxicity release (Fig. 2) in Caco-2 cells that were treated for 12 and 24 h with CF extracts was lowest at the highest concentration (400  $\mu\text{g mL}^{-1}$ ). The LDH release reported for 12 h for ranged from 8.1-45.8% and for 24 h it ranged form a low of 20% to a high of 60%. Highest LDH release (% cytotoxicity) after 12 and 24 h was seen at concentrations of 25  $\mu\text{g mL}^{-1}$  (60%) and 50  $\mu\text{g mL}^{-1}$  (44%).

**Enzymatic activities:** The GSH content (nmol  $\text{mg}^{-1}$  protein) in Caco-2 cells was significantly higher ( $p < 0.05$ ) at concentrations of 0 and 25  $\mu\text{g mL}^{-1}$  (Fig. 3). Highest GSH levels were at a concentration of 0  $\mu\text{g mL}^{-1}$  (control) and the lowest GSH content was observed at 50  $\mu\text{g mL}^{-1}$  (6.49 nmol  $\text{mg}^{-1}$ ). As observed in GSH activity no significant differences were reported in GST activity. However, highest GST activity (Fig. 4b) was observed at 25  $\mu\text{g mL}^{-1}$  and the lowest GST activity was observed at 100  $\mu\text{g mL}^{-1}$  compared to the control (0  $\mu\text{g mL}^{-1}$  (1.95 $\pm$ 0.19)). Glutathione peroxidase (GPx) activities (nmol  $\text{min}^{-1}$   $\text{mL}^{-1}$ ) observed in Caco-2 cells treated with CF ranged from a low of 14.51 (50  $\mu\text{g mL}^{-1}$ ) to a high of 20.84 (0  $\mu\text{g mL}^{-1}$ ). The CAT activity (Fig. 4c) ranged from

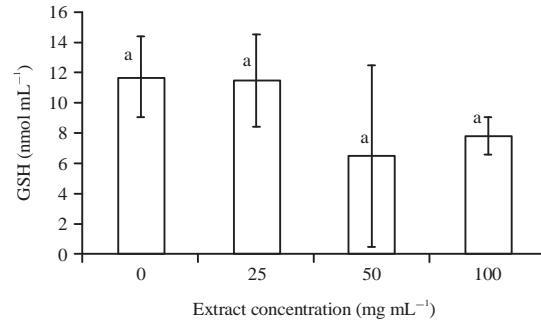


Fig. 3: Glutathione (GSH) levels of Caco-2 cells treated with Coconut Flour (CF) extracts for 24 h. Bars are means. <sup>abc</sup>Bars not sharing common superscript are significantly different ( $p < 0.05$ ) by Tukey's studentized range test

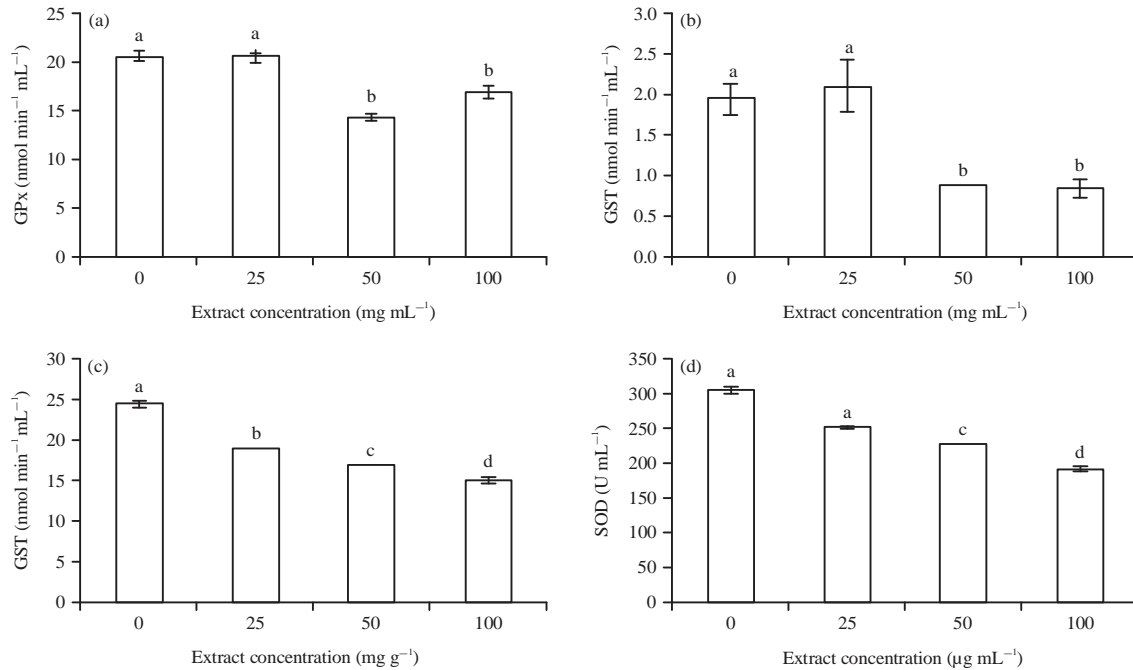


Fig.4(a-d): Enzymatic activity of antioxidant and detoxification enzymes, (a) Glutathione peroxidase (GPx) activity of Caco-2 cells treated with CF extract, (b) Glutathione-s-transferase (GST) activity of Caco-2 cells treated with CF extract, (c) Catalase (CAT) activity of Caco-2 cells treated with CF extracts and (d) Superoxide dismutase (SOD) in Caco-2 cells treated with Coconut Flour (CF) extracts for 24 h

a low of 5.25 U mg<sup>-1</sup> to a high of 24.65 U mg<sup>-1</sup>. Among concentrations highest CAT activity was at a concentration of 25 µg mL<sup>-1</sup>. Through the dismutase action of SOD, highest activity among concentrations (25-100 µg mL<sup>-1</sup>) was also observed at 25 µg mL<sup>-1</sup> (Fig. 4d). Significant differences were observed in SOD activity, which ranged from low of 192 U mL<sup>-1</sup> to a high of 305 U mL<sup>-1</sup>. There were no significant difference in Cox-2 activity of Caco-2 cells treated with CF extracts between the control (0.27 U mL<sup>-1</sup>) and the extract concentrations of 25 (0.30), 50 (0.25) and 100 (0.20). However, with an increase in concentration there was a decrease in Cox-2 activity.

**Cellular DNA fragmentation ELISA and caspase-3 activity:** Results for cellular DNA fragmentation ELISA were reported as Enrichment Factor (EF). The EF of cells treated with CF ranged from 0.27-0.39. There were no significant ( $p < 0.05$ ) differences among concentrations but the highest EF was reported at a concentration of  $50 \mu\text{g mL}^{-1}$  (0.398). Caspase-3 was significantly ( $p < 0.05$ ) higher in comparison to the control in cells treated with CF extracts at concentration of  $100 \mu\text{g mL}^{-1}$ .

## DISCUSSION

The Total Phenolic and Total Flavonoid Content (TPC and TFC) of Coconut Flour (CF) extracts is shown in Table 1. An increase in temperature has been shown to increase phenolic content in coconut by products (Rodrigues *et al.*, 2008). At higher temperatures, more phenolic compounds can be extracted from coconut oil (Seneviratne and Dissanayake, 2008). The TFC (CE/100 g) defatted -25°C CF showed a significant ( $p < 0.05$ ) increase, these results were higher than what was reported by Sani *et al.* (2014) in coconut oil extracted with a soxhlet apparatus. In the present study n-hexane was utilized as the solvent for lipid extraction and for whole extraction 80% methanol was used. The type of solvent and extraction methods can affect the presence of flavonoid compounds (Andersen and Markham, 2006). Alothman *et al.* (2009) found that the type of solvent utilized (acetone, methanol and ethanol) affected the extractability of flavonoid compounds in pineapple, banana and guava extracts; showing that the use of methanol can significantly increased the total flavonoid content with respects the type of fruit (e.g., honey pineapple, banana *Pisang mas*).

The free radical (DPPH) scavenging activity of CF extracts exhibited a concentration dependence (Fig. 1) in the inhibition of DPPH radical, which ranged from 2-60%. In a study conducted by Santos *et al.* (2013), an  $\text{IC}_{50}$  (DPPH) of  $150 \mu\text{g mL}^{-1}$  for a common variety of coconut water was reported. In this present study an  $\text{IC}_{50}$  (Table 3) was observed at lower concentrations for the CF extracts of whole -25°C, defatted -25°C and defatted -170°C. Although, defatted -170°C extracts did not scavenge the DPPH radical at an  $\text{IC}_{50}$ , the results show that the defatted -170°C extracts have scavenging ability with increasing concentrations. After the inhibition at a concentration of  $80 \text{ mg mL}^{-1}$ , a steep decrease in DPPH radical was observed for all CF extracts. Alothman *et al.* (2009) reported a significant percentage DPPH inhibition in methanol extracts compared to acetone and ethanol fruit extracts.

The antioxidant potential of CF extracts to scavenge for the 2,2'-azinobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) was assessed by Trolox Equivalent Antioxidant Capacity (TEAC) assays (Table 3). More specifically, CF extracts in defatted -25°C ( $0.65 \pm 0.05$ ), contained the highest total phenolic and flavonoid content (Table 1), which may have contributed to, increased scavenging of the ABTS radical. It is possible that the higher ferric reducing antioxidant power (Table 3) of CF extracts value observed in whole and defatted -25°C compared to whole and defatted -170°C was due to a temperature effect. The lower temperatures yielded a higher FRAP value compared to the

Table 3: 1, 1-diphenyl-1-picrylhydrazyl (DPPH) in coconut flour extracts

DPPH $\text{IC}_{50}$ ( $\text{mg mL}^{-1}$ )	
Treatments	CF
Whole -25°C	74
Whole -170°C	80 ( $\text{IC}_{30}$ )
Defatted -25°C	72
Defatted -170°C	62

Whole and defatted: Extraction at temperatures of 25 and 170°C, DPPH: 2, 2-Diphenyl-1-picrylhydrazyl, CF: Coconut flour



Table 4: Caspase-3 activity in Caco-2 cells treated with coconut flour extracts

Concentrations ( $\mu\text{g mL}^{-1}$ )	Caspase-3
0	1.00 $\pm$ 0.02 <sup>b</sup>
25	1.52 $\pm$ 0.05 <sup>c</sup>
50	1.53 $\pm$ 0.05 <sup>b</sup>
100	1.82 $\pm$ 0.03 <sup>a</sup>

Values are Mean $\pm$ SD, n = 3. <sup>abc</sup>Values not sharing common superscript are significantly different (p<0.05) using Tukey's studentized range test

CF extractions at 170°C. Oxygen-Radical Absorbance Capacity (ORAC) assay was used to assess the ability of the CF extracts to prevent further oxidative damage of the fluorescein molecule, which is evident, by an increase in fluorescence reading (Table 3) as observed in whole -170°C CF which was significantly (p<0.05) higher among extraction methods.

The LDH percent cytotoxicity release (Fig. 2) in Caco-2 cells for 12 h at a concentration of 50  $\mu\text{g mL}^{-1}$  the CF extracts affected the cellular integrity of the Caco-2 colon cancer cells, causing the release of LDH in response to cellular cytotoxicity. Cytotoxic effects were similar at concentrations of 50 and 200  $\mu\text{g mL}^{-1}$  for 24 h and 25 and 100  $\mu\text{g mL}^{-1}$  for 12 h. Based on these results the most effective concentrations for the effect of CF extracts on selective antioxidant and detoxification enzymes were determined. The percent cytotoxicity at 12 and 24 h was concentration dependent with the highest LDH release reported at concentrations between 25 and 100  $\mu\text{g mL}^{-1}$ .

Reduced-S-glutathione (GSH) functions as a nucleophilic co-substrate to Glutathione-S-Transferase (GST) and Glutathione Peroxidases (GPx) in the detoxification of harmful peroxides (Fig. 3) (Griffith, 1985). Although there were no significant differences in GSH levels among concentrations, highest GSH levels were at a concentration of 0  $\mu\text{g mL}^{-1}$  (control) and the lowest GSH content was observed at 50  $\mu\text{g mL}^{-1}$  (6.49 nmol mg<sup>-1</sup>). Glutathione Peroxidase (GPx) activities (nmol min<sup>-1</sup> mL<sup>-1</sup>) observed in Caco-2 cells treated with CF ranged from a low of 14.51 (50  $\mu\text{g mL}^{-1}$ ) to a high of 20.84 (0  $\mu\text{g mL}^{-1}$ ). Among concentrations, highest GPx activity was at 25  $\mu\text{g mL}^{-1}$ . The GST and GPx results are consistent with the GSH findings reported. There was no significant difference between the control and GPx activity (Fig. 4a). Because reduce GSH (Fig. 3) functions as a co-substrate for GST and GPx, enzymatic activity was highest at 25  $\mu\text{g mL}^{-1}$  among concentrations. At higher concentrations a decrease in enzymatic activity was observed (Fig. 3 and 4a, b). Catalase (CAT) activity decreased with increasing concentrations of incubation with CF extracts (Fig. 4c). The CF extracts increased the action of catalase neutralizing hydroperoxide to water and oxygen. In addition CF extracts also increased the SOD dismutation activity of hydrogen peroxide at a concentration of 25  $\mu\text{g mL}^{-1}$ .

Synthesis of prostaglandins and other eicosanoids is possible through the activity of cyclooxygenase (Cox-2), which is often expressed due to an inflammatory or immune response (Crofford, 1997). No significant difference (p<0.05) in Cox-2 activity was seen in cells treated with various concentrations of CF extracts (Fig. 4).

Apoptotic activity reported as expression of Cellular DNA Fragmentation and Caspase-3 of Caco-2 cells and treated CF extracts exhibited concentration dependence in relation to the control (Table 4, Fig. 4). Results for Cellular DNA fragmentation ELISA were reported as Enrichment Factor (EF). The EF of cells treated with CF ranged from 0.27-0.39. There were no significant (p<0.05) differences among concentrations but the highest EF was reported at a concentration of 50  $\mu\text{g mL}^{-1}$  (0.398). There were significant (p<0.05) differences in Caspase-3 activity in cells treated with CF extract for 24 h among concentrations (0, 25, 50 and 100  $\mu\text{g mL}^{-1}$ ).

The interest in antioxidants as a form of chemoprevention is increasing (Bagchi *et al.*, 2014; Singh, 2014; Klotz, 2014; Danaraddi *et al.*, 2014). From this study it was concluded that extraction temperature affects the extractability of total phenolics and flavonoids. Furthermore, this effect was evident in the ability of the CF extracts to scavenge for free radicals as expressed by the antioxidant assays TEAC, FRAP, ORAC and DPPH. Increased activity of antioxidant and detoxification enzyme activity suggests the effectiveness of CF extracts to enhance detoxifying enzymes within the body. Further *in vivo* studies are needed to investigate the anti-carcinogenic effects of CF extracts on colon cancer within animal models.

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