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Bioactivity Studies of Different Solvent Extracts of Partially Defatted Coconut Testa Obtained from selected Coconut Cultivars

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ABSTRACT

Purpose: *Coconut testa is a byproduct of coconut industry and is currently used for oil extraction. The partially defatted residue of testa is underutilized despite being able to convert into coconut testa flour (CTF). The objective of the present study was to compare the bioactivity of different solvent extracts of CTF obtained from selected Sri Lankan coconut cultivars namely, Gon Thambili (GT), Ran Thambili (RT), San Raman (SR), Tall×Tall (TT) and commercial hybrid (COM).*

Research Method: *CTF of individual cultivars was sequentially extracted with hexane, ethyl acetate (EtOAc) and methanol (MeOH). The total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), DPPH radical scavenging activity, alpha amylase (Alpha-amy) and alpha-glucosidase (Alpha-glu) inhibitory activities were established in vitro using relevant assays. The phytochemical profiling of CTF was done using ultra high pressure liquid chromatography coupled with mass spectrometry.*

Findings: *Among solvent extracts, MeOH extracts of all cultivars displayed significantly higher TPC, TFC, antioxidant and Alpha-amy inhibitory activities. GT exhibited the highest TPC (102.48 ± 3.46 GAE/g of crude extract), TFC (63.49 ± 4.47 CE/g of crude extract), FRAP value (1097.23 ± 1.24 µmol FeSO4 /g of crude extract), DPPH radical scavenging activity (IC50, 45.37 ± 1.94 ppm) while TT resulted in the highest Alpha-amy inhibitory activity (IC50, 80.09 ± 4.67 ppm). The EtOAc extract of TT showed the highest Alpha-glu inhibitory activity (IC50, 7.82 ± 0.40 ppm). The distribution of phenolic constituents was found to vary among the different cultivars.*

Originality/value: *This study concludes that the extracts of CTF is a potent source of bioactive compounds that claim various bioactivities which can be used in developing functional foods.*

Keywords: *Antioxidant activity, Anti-hyperglycemic activity, Bioactivities, Coconut testa flour*

INTRODUCTION

Coconut is one of the major types of plantation crops, which contributes about 12 % of all agricultural produces in Sri Lanka. Coconut provides a number of products out of various parts of the tree. Coconut kernel is the most important component of its fruit, which is used to make various products such as coconut oil, desiccated coconut, virgin coconut oil, coconut milk powder and coconut milk. Coconut testa (CT) is the brown outer layer which covers the white kernel and constitutes about 18 % of the total wet weight of coconut kernel (Marasinghe *et al*., 2019). Since CT could impart a brown color to the oil and dull appearance to other products, it is usually removed as a byproduct

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during the production of various kernel-based products. As Sri Lanka's annual national coconut production is around 2,450 million nuts (CDA 2019), there is proportionally a high amount of CT being remained as a byproduct. In general, the CT is used to extract low-grade oil and the remaining residue is used as a feedstock for animals. At present, the commercial use of CT considering its active compounds and nutritional benefits is limited. However, the utilization of this agricultural by product is encouraged to help minimize the environmental impact and generate extra revenue from coconut processing industries (Doshi *et al*., 2015).

In a preliminary effort to utilize coconut testa in a beneficial way, Marasinghe *et al.* (2019) evalauted the proximate composition of partially defatted coconut testa flour produced from local coconut cultivars such as Gon Thambili, Ran Thambili, San Raman, Tall×Tall and commercial hybrid. As coconut testa flour (CTF) is basically a product prepared from the coconut testa residue left after oil extraction, it is rich in protein which is twice the protein content of commercial wheat flour (Marasinghe *et al.*, 2019). Hence, the suitability of CTF for partial substitution of wheat flour in cookie preparation was tested in a subsequent study, which yielded promising results (Marikkar *et al.,* 2020). However, the evaluation of the bioactivities such as antioxidant, anti-hyperglycemic effects of CTF produced from locally grown coconut cultivars was rarely undertaken. The assessment of these bioactivities would be useful to promote CTF as a nutritional raw material for development of functional foods. Hence, the objective of this study was to screen for bioactivities of coconut testa flour obtained from different Sri Lankan coconut cultivars namely, GT, RT, SR, TT, and COM.

MATERIALS AND METHODS

Plant Materials

Coconuts of twelve-month maturity were collected from five different local cultivars (i.e. GT, RT, SR, TT, and COM) that were maintained at the varietal blocks of Coconut Research Institute, Lunuwila, Sri Lanka. Fifty nuts of each cultivar was collected during the time period of November 2019 to February 2020. Preparation of coconut testa flour (CTF) was done according to the flow chart described by Marasinghe *et al.* (2019). Briefly, seasoned coconuts were subjected to de-husking, de-shelling and de-paring. The testa removed from the kernel was disintegrated before putting into the drying oven. Dried testa was subjected to cold oil extraction and the remaining residue (partially defatted coconut testa) was ground to make CTF. Prepared CTF was kept at 4 °C for further analysis.

Reagents and Instruments

Enzymes; Porcine pancreatic Alpha-amy, Alpha-glu (from *saccharomyces cerevisiae*) and fourteen phenolic standards namely; catechin, caffeic acid, gallic acid, ferulic acid, chlorogenic acid, ellagic acid, sinapic acid, p-coumaric acid, vanillic acid, epigallocatechin gallate (EGCG), quercetin, rutin, epicatechin, kaempferol were purchased from Sigma-Aldrich. All other chemicals used in the experiments were analytical grade unless otherwise specified. The UV absorbance measurements were taken using a microplate reader (Synergy HTX Biotek Multimode reader, Biotek instruments USA).

Preparation of Crude Extracts

A 250 g of CTF of each cultivar (GT, RT, SR, TT and COM) was sequentially extracted with hexane, EtOAc and MeOH by sonicating (Rocker ultrasonic cleaner, model-Soner 206H) for 30 min. In each solvent, extraction was repeated three times. The extracts were concentrated in a rotary evaporator (Heidolph, Laborota 4000) and the obtained crude extracts were freeze dried (ESCO, model-FDL-2S8, Singapore). The samples were stored at -18˚C until further analysis.

Determination of Total Phenolic Content

TPC of CTF was assayed by following Folin-Ciocalteu (FC) method reported by Alyaqoubi *et*

al. (2015) with slight modifications. A fifty µL portion of the solution (reconstituted crude extract with distilled water), 15 µL of distilled water, 105 µL of 10 % FC reagent were added to 96 well micro-plate and 80 µL of 7.5 % Na_2CO_3 was added after 3 min. After incubating the content of the mixture for 30 min at room temperature (RT) in dark conditions the absorbance value was recorded at 765 nm. TPC of crude extracts were expressed as mg gallic acid equivalent (GAE) per g of crude extract.

Determination of Total Flavonoid Content

TFC of CTF was analyzed by following the aluminum chloride colorimetric method described by Adekola *et al.* (2017) with slight changes. A fifty µL aliquots of sample solution (reconstituted crude extract with distilled water) was mixed with 20 µL of 5 % $NaNO_2$ in 96 well micro-plate, incubated for 6 min at RT and 20 µL of 10 % $AlCl_3$ was added. A 200 µL of 4 % NaOH solution was added after another 6 min. After an incubation period of 15 min, the absorbance values were recorded at 510 nm. TFC was expressed as mg catechin equivalent (CE) per g of crude extract.

Determination of DPPH Radical Scavenging Activity

This assay was carried out by following the method reported by Alakolanga (2015) and Tepe *et al.* (2007). Briefly, a concentration series of crude extracts were prepared by reconstituting crude extract in MeOH and added with 150 µL aliquots of each concentration solution into 60 µL of 0.3 mM DPPH solution in 96 well micro-plate, allowed for 30 min in dark at RT. The absorbance values were recorded against the control at 517 nm. Both ascorbic acid and butylated hydroxyl anisole (BHA) were used as positive controls. The % inhibition was calculated as below and IC_{50} value was calculated graphically.

$$RSA\% = \frac{\delta A\ control - \delta A\ sample}{\delta A\ control} \times 100$$

Where; $\delta A_{control} = Absorbance_{control} - Absorbance_{control\ blank}$, $\delta A_{sample} = Absorbance_{sample} - Absorbance_{sample\ blank}$; RSA %, Percentage of radical scavenging activity.

Determination of Ferric Reducing Antioxidant Power

This assay was performed by adopting the method described by Abubakar *et al*. (2017) with minor modifications. A fifty µL of aliquot of sample solution (reconstituted crude extract with distilled water) was added to 96-well microplate and mixed with 150 µL of FRAP solution and allowed to incubate for 4 min at RT. The absorbance values were recorded at 593 nm and the values were expressed as µmol $FeSO_4$ per g of crude extract. In this experiment, both ascorbic acid and BHA were used as positive controls. FRAP solution was prepared by mixing 10 mM TPTZ solution (in 40 mM HCL), 10 mM $FeCl_3.6H_2O$ solution with 40 mL of 300 mM, pH 3.6 acetic buffer in the ratio of 1:1:10 and was subjected to heating up to 37 °C just prior to use.

Enzyme Inhibitory Assays

Alpha amylase inhibition: Alpha-amy inhibitory activity of CTF crude extracts was done by following the method described by Nickavar *et al.* (2008) and Alakolanga (2015) with minor modifications. A concentration series of crude extracts were prepared in deionized water. A fifty µL of each solution was mixed with 50 µL of enzyme solution (20 mg/ mL) in a semi micro centrifuge tube and incubated for 30 min at 25 °C. After adding 100 µL of 0.5 % starch solution, the mixture was incubated for another 3 min at 25 °C followed by adding 100 µL of DNSA (3,5-dinitrosalicylic acid) reagent. Thereafter, the mixture was incubated at 85 °C for 15 min in a water bath. The mixture in the semi centrifuge tube was allowed to cool, diluted with 900 µL of deionized water and the absorbance was recorded at 540 nm. In this experiment, acarbose was used as the positive control. After plotting the Alpha-amy % inhibition against sample concentration, the IC_{50} values were obtained graphically.

$$Percentage\ Inhibition\ of\ \alpha - amylase\ activity = \frac{\delta A\ control - \delta A\ sample}{\delta A\ control} \times 100$$

Where; $\delta A_{control} = Absorbance_{control} - Absorbance_{control\ blank}$, $\delta A_{sample} = Absorbance_{sample} - Absorbance_{sample\ blank}$

Alpha glucosidase inhibition: Alpha-glu inhibitory activity of CTF crude extracts was carried by adopting the method described by Sambavathas (2019) with minor modifications. The assay was performed in 96-well microplate. A concentration series of crude extracts was prepared in deionized water. A hundred µL of 30 mM (pH 6.5) phosphate buffer was added to wells followed by adding 25 µL of sample solution (reconstituted crude extract with deionized water). Subsequently, 25 µL of Alpha-glu enzyme solution (12.5µL/mL) was added, followed by incubation at 37 °C for 5 min. After adding 50 µL of pNPG solution (0.8 mg/mL), it was again incubated at 37 °C for 30 min and absorbance was recorded at 410 nm. In this experiment, acarbose was used as the positive control. Calculation of the % inhibition of Alpha-glu was based on the following equation. After plotting % inhibition vs. the sample concentration, the IC_{50} value was calculated graphically.

$$Percentage\ Inhibition\ of\ \alpha-glucosidase\ activity = \frac{\delta A\ control - \delta A\ sample}{\delta A\ control} \times 100$$

Where; $\delta A_{control} = Absorbance_{control} - Absorbance_{control\ blank}$, $\delta A_{sample} = Absorbance_{sample} - Absorbance_{sample\ blank}$

Phyto-chemical profiling by LC-MS

LC-MS analysis of CTF was carried out following the method described by Alakolanga (2015) with modifications. A five g sample of CTF of each cultivar was dissolved in 25 mL portion of 70 % aqueous methanol (HPLC grade methanol, >99.99 % in ultrapure water) and sonicated for 30 min. The extract was filtered through a syringe filter (25 mm, 0.45 um) and the filtrate was used for the LC-MS analysis.

The system used was an ultra-high pressure liquid chromatograph (UHPLC) (UltiMate™ 3000, Thermo Scientific, Germany) equipped with a quaternary pump (LPG-3400SD), auto sampler (ACC-3000) and diode array detector (DAD-3000) which was set to record signals at 224 nm, 254 nm, 280 nm and 360 nm wavelengths. An iron trap mass spectrometer (LCQ FLEET, Thermo Scientific, USA) fitted with as electrospray ionization (ESI) source operating in full scan, auto MS^n mode was coupled to the system to obtain fragment ion m/z. A 10 µL of the sample was injected to the system fitted with a Ascentis RP – Amide column (5 µm) (Supelco Analytical 15 cm x 4.6 mm, Merck, Germany). A mixture of methanol (A) and acidic water containing 0.01 % formic acid (B) was the mobile phase, where solvents were delivered to the system in a gradient elution at a flow rate of 0.400 mL/min. The gradient program was arranged as; 90 % of solvent B from 0-5min, 90-2 % of solvent B from 5-65 min, 2 % of solvent B from 65-70 min, 2-90 % of solvent B from 70-75 min, 90 % of solvent B from 75-80 min. The MS spectra was obtained in negative iron mode and the parameters were set as below; heat temperature, 350 ºC, sheath N_2 gas flow rate, 36 arbitrary units; aux N_2 gas flow rate, 9 arbitrary units; spray voltage, 4.50 kV; capillary temperature, 320 ºC; capillary voltage, -40.00 V; tube lens, -95.00 V. The mass chromatogram was recorded from 110-1500 m/z. For qualitative detection and quantitative analysis, 13 phenolic compounds were used as authentic standards while calibrating the same setting source parameters.

Statistical Analysis

Triplicate analyses were performed (n=3) and the results were presented as mean ± standard deviation (SD). Experimental results were analyzed by one-way ANOVA using Minitab 17 software package. When the F values were significant, mean differences were compared using Tukey's test at the 5 % significance level. The correlation between bioactivities and the strength of the relationship was indicated by performing Pearson's linear correlation at 5 % significance level.

RESULTS AND DISCUSSION

Total Phenolic Content

Phenolics, a type of natural compounds occurring in plants, are structurally different and vary from simple phenolic acid to polyphenols (Cheynier, 2012). According to data shown in Table 01, inter-

varietal difference in TPC contents was observed among different solvent extracts. Among different cultivars, MeOH extracts resulted in the highest TPC values while hexane and EtOAc extracts resulted in the lowest values. Previously, Barchan *et al*. (2014) mentioned that methanol was the best organic solvent to extract phenolic compounds from plant materials. According to Ojha *et al.* (2019), sequential extraction process would help to enhance the extractability of phenolic compounds in the MeOH fraction. Therefore, the high TPC in MeOH extract of CTF could be assumed to be due to occurrence of large amount of highly polar phenolic constituents (Babbar *et al*., 2014). The TPC of MeOH fractions varied from 15.69 ± 0.20 to 102.48 ± 3.46 mg GAE per gram of crude extract in the descending order of GT >TT >SR >RT >COM. According to statistical analysis, the values were significantly ($p < 0.05$) different except those of GT and TT. Among some cultivars, negative results were obtained for fractions, namely, hexane extract of COM and EtOAc extracts of GT and SR. The TPC for hexane extracts was found to align in the descending order of GT >TT > SR > RT where the highest and the lowest values were 3.09 ± 0.00 and 0.92 ± 0.16 mg GAE /g of crude extract, respectively. Except for GT and TT, the values were significantly ($p < 0.05$) different. TPC of EtOAc varied from 0.41 ± 0.10 to 4.40 ± 0.43 mg GAE /g of crude extract and followed the order of RT >COM >TT where only TT was significantly ($p < 0.05$) different.

Total Flavonoid Content

The mean values of TFC for different crude extracts obtained using different solvents are shown in Table 01. Among the extracts, only MeOH extracts resulted in the highest TFC for all five cultivars. Out of the different extracts obtained using EtOAc and hexane, only hexane extract of GT displayed a slight amount of TFC while other extracts did not show any positive result at all. This finding was in conformity with those findings reported by other works. Previously, Ojha *et al.* (2019) stated that high flavonoid contents were only presented in MeOH extract but not in other solvent extracts obtained using petroleum benzene, ethyl acetate, chloroform, water, etc. Similarly, Adekola *et al.* (2017) noticed that 70 % ethanol-water extracts of coconut testa had a high flavonoid content. In a separate study, Appaiah *et al.* (2016) claimed that the highest TFC was in ethanolic extracts of wet coconut testa and copra testa. TFC of MeOH extracts was found to be within the range of 6.42 ± 0.37 - 63.49 4.47 CE (mg) / g of crude extract with GT exhibiting the highest value and COM showing the lowest value. Although TFC values followed the order of GT> TT> SR> RT> COM, no significant ($p > 0.05$) difference between GT and TT was noticed.

Table 01: Total phenolic and flavonoid contents of hexane, ethyl acetate and methanol extracts obtained from CTF of selected cultivars

Assay	Cultivar	Type of Extract		
		Hexane	EtOAc	MeOH
Total phenolic content (mg gallic acid equivalent (GAE) /g of crude extract)	COM	nd	4.40 ± 0.43^{b}	15.69 ± 0.20^{a}
	GT	3.09 ± 0.00^{c}	nd	102.48 ± 3.46^{d}
	RT	0.92 ± 0.16^{a}	4.68 ± 0.17^{b}	53.32 ± 1.19^{b}
	SR	1.81 ± 0.16^{b}	nd	90.11 ± 0.84^{c}
	TT	2.62 ± 0.23^{c}	0.41 ± 0.10^{a}	102.16 ± 1.98^{d}
Total flavonoid content (mg catechin equivalent (CE) /g of crude extract)	COM	nd	nd	6.42 ± 0.37^{a}
	GT	1.58 ± 0.14	nd	63.49 ± 4.47^{d}
	RT	nd	nd	28.79 ± 0.74^{b}
	SR	nd	nd	39.98 ± 0.35^{c}
	TT	nd	nd	58.78 ± 2.08^{d}

Each value in the Table 0represents the mean of three replicates. In each assay, the means that do not share a similar simple superscription letter within the columns are significantly different at 95 % confident (α=0.05). Abbreviations: COM, commercial hybrid; GT, Gon Thembili; RT, Ran Thembili; SR, San Ramon; TT, TallxTall; nd, not detected; wk, weak activity: IC50, half maximal inhibitory concentration.

Phenolic profile of extracts

The results in Table 02 show the quantitative distribution of phenolic constituents present in CTF. According to Table 02, out of the 13 authentic standards, compounds including caffeic acid, chlorogenic acid, ellagic acid, p-coumaric acid, vanillic acid, epigallocatechin gallate (EGCG), quercetin and rutin were present in all cultivars. Compounds namely, epicatechin and kaempferol were not detected in any of the five cultivars. Among the detected compounds, chlorogenic was the most predominant phenolic acid in all cultivars. It was ranged between 145.52 - 49.50 mg/100 g where GT and COM were recorded the highest and the lowest values, respectively. Among the cultivars, the values were significantly ($p < 0.05$) different and can be aligned in the order of COM< RT< TT< SR< GT. Previously, Adekola *et al.* (2017) were also able to detect roughly similar amount of chlorogenic acid in the whole testa of Malaysian coconut cultivar. Chlorogenic acid occurring in fruits, vegetables and cereals is generally known to play an important role as an antioxidant, anti-inflammation and antihypertension agent. According to several research studies, it has the potential to reduce the organ damages and entail hepato-protective effects in animals (Naveed *et al*., 2018).

The data presented in Table 02 show that ellagic acid was the second most abundant phenolic constituent followed by vanillic acid. The content of ellagic acid varied from 11.49 mg/100 g to 11.65 mg/100 g where the lowest and highest values were possessed by GT and COM, respectively. Among the cultivars, the values did not show a significant ($p > 0.05$) difference. Ellagic acid and its derivatives occurring in fruits, vegetables and cereals exert various health promotive physiological activities mainly; antioxidant, antimicrobial, antiglycative, anti-inflammatory, estrogenic and/or antiestrogenic and prebiotic activities. García-Niño and Zazueta (2015) stated that ellagic acid has the ability to improve the hepato-protective functions due to its antioxidant, anticholestatic, antisteatosic, antihepatocarcinogenic, antifibrogenic and antiviral properties.

Table 02: Distribution of selected phenolic constituents present in methanol extract of coconut testa flour

Phenolic compound	Content (mg/100g of CTF)				
	COM	GT	RT	SR	TT
Caffeic acid	1.50 ± 0.00^{b}	1.60 ± 0.00^{c}	1.34 ± 0.01^{a}	1.40 ± 0.01^{a}	1.51 ± 0.01^{b}
Chlorogenic Acid	49.50 ± 0.00^{a}	145.52 ± 0.01^{e}	76.92 ± 0.00^{b}	131.83 ± 0.01^{d}	114.90 ± 0.01^{c}
Gallic acid	2.13 ± 0.01^{a}	2.16 ± 0.01^{a}	2.13 ± 0.01^{a}	2.14 ± 0.01^{a}	nd
Ellagic acid	11.65 ± 0.01^{a}	11.49 ± 0.01^{a}	11.50 ± 0.00^{a}	11.51 ± 0.01^{a}	11.50 ± 0.01^{a}
Ferulic acid	0.28 ± 0.01^{a}	Nd	0.29 ± 0.00^{a}	0.31 ± 0.01^{a}	nd
p-Coumaric acid	0.49 ± 0.00^{a}	0.62 ± 0.00^{b}	0.49 ± 0.01^{a}	0.73 ± 0.01^{c}	0.72 ± 0.01^{c}
Sinapic acid	nd	Nd	nd	nd	1.61 ± 0.01^{a}
Vanillic acid	7.86 ± 0.01^{d}	7.72 ± 0.00^{d}	6.36 ± 0.01^{a}	6.78 ± 0.00^{b}	7.12 ± 0.01^{c}
Epigallocatechin gallate	2.73 ± 0.01^{a}	3.28 ± 0.00^{c}	3.00 ± 0.01^{b}	3.26 ± 0.00^{c}	3.09 ± 0.00^{b}
Epicatechin	nd	Nd	nd	nd	nd
Quercetin	6.32 ± 0.01^{a}	6.33 ± 0.01^{a}	6.34 ± 0.01^{a}	6.37 ± 0.01^{a}	6.35 ± 0.00^{a}
Rutin	1.92 ± 0.00^{b}	1.45 ± 0.01^{a}	1.50 ± 0.01^{a}	1.52 ± 0.01^{a}	1.57 ± 0.01^{a}
Kaempferol	nd	Nd	nd	nd	nd

Each value in the Table 0represents the mean of replicates. Means that do not share a similar simple superscription letter within the rows are significantly different at 95 % confident (α=0.05). Abbreviations: COM, commercial hybrid; GT, Gon Thembili; RT, Ran Thembili; SR, San Ramon; TT, TallxTall; nd, not detected.

According to Table 02, vanillic acid ranged between 6.36 mg/100 g for RT and 7.86 mg/100 g for COM. The values can be arranged in the order of RT< SR< TT< GT< COM and significant ($p < 0.05$) differences were found among RT, SR and TT. According to Kim *et al.* (2010), vanillic acid occurring in fruits, vegetables and cereals can be effectively engaged in the management of inflammatory responses.

Gallic and caffeic acids were the next most abundant phenolics present in CTF after vanillic acid. According to Table 02, gallic acid was detected in all cultivars except TT. Values ranged from 2.13 mg/100 g to 2.16 mg/100 g where the lowest and the highest values were recorded for COM and GT, respectively. The values, however, did not show any significant ($p > 0.05$) difference. The previous scientific evidence showed that gallic acid and its derivatives occurring in fruits, vegetables and cereals could be effective in various pharmacological activities such as; anti-inflammatory, anti-gastrointestinal, anti-cardiovascular etc. The pharmacological activities of gallic acid is mostly pertaining to its antioxidant and anti-inflammatory mechanism (Kahkeshani *et al.*, 2019). According to Table 02, caffeic acid content of the extracts was found to vary between 1.34 mg/100 g for RT and 1.60 mg/100 g for GT. Among the cultivars, there was hardly any significant ($p > 0.05$) difference. Based on the previous in-vivo and in-vitro studies, caffeic acid and its derivatives occurring in fruits, vegetables and cereals were known to possess interesting physiological properties such as; antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticarcinogenic, anti-atherosclerotic, hepatoprotective, immunostimulatory, cardioprotective and anti-hepatocellular carcinoma activities (Monteiro Espíndola *et al.*, 2019).

Among the cultivars, sinapic acid was detected only in TT cultivar but the amount was about 1.61 mg/100 g. This acid occurring in fruits, vegetables and cereals has been known to exhibit antioxidant, antiglycemic, anticancer, anti-inflammatory, antimutagenic, neuroprotective and antibacterial potentials (Chen, 2016). Among the cultivars, p-coumaric acid was found in comparatively low amounts where the values were varied between 0.49 mg/100 g for RT and 0.73 mg/100 g for SR. However, any significant ($p > 0.05$) differences were not found among COM, RT as well as SR, TT. In the case of ferullic acid, it was only detected in COM, RT, SR cultivars; the highest value was recorded for SR (0.31 mg/100 g) while the lowest value was noted for COM (0.28 mg/100 g). The values, however, did not show any significant ($p > 0.05$) difference. According to Sen *et al.* (2013), p-coumaric and ferulic acids occurring in fruits, vegetables and cereals were known to possess antioxidant, anti-tumor, anti-viral, anti-fibrosis, anti-thrombotic potentials. Further, ferulic acid has the potential to exert pharmacological activities against diseases such as; diabetes, cancer, neuro-generative and cardiovascular diseases (Srinivasan *et al.*, 2007). As shown in Table 02, quercetin was the most abundant flavonoid found in the samples (between 6.32 mg/100 g for COM to 6.37 mg/100 g for SR). Nevertheless, the values observed for different cultivars did not show any significant ($p > 0.05$) difference. Based on the previous literature, quercetin occurring in fruits, vegetables and cereals was known to promote overall physical and mental health as well as exert some other beneficial health properties due to its antioxidant, anti-inflammatory, anti-carcinogenic, antiviral and psychostimulant activities (Li *et al.,* 2016). Quercetin is also reported to be effective against series of diseases such as diabetic complications (including diabetic liver disorders, nephropathy, reproductive and neuro-degenerative disorders, retinopathy), cardiovascular diseases, arthritis, liver diseases, alzheimer's disease (Salehi *et al.* 2020).

EGCG was the next most abundant flavonoid in the extracts and found to vary between 2.73 mg/100 g for COM and 3.28 mg/100 g for GT. However, there was hardly any significant ($p > 0.05$) difference found among the values obtained for different cultivars. EGCG occurring in fruits, vegetables and cereals is famed for its antioxidant, chemo prevention, cardiovascular health promotion, weight management (Judul and Pendapatan, 2015) and neuroprotective potential (Singh *et al.,* 2016). Among the extracts of different cultivars, rutin was found to range between 1.45 mg/100 g for GT and 1.92 mg/100

g for COM where COM showed a significant ($p < 0.05$) difference compared to the other cultivars. According to previous literature, rutin is famed for a wide range of pharmacological benefits such as, antioxidant, cardioprotective, cytoprotective, anticarcinogenic, vasoprotective and neuroprotective (Ganeshpurkar and Saluja, 2017).

The phenolic and flavonoid distributions of the whole coconut test were previously reported for Malaysian (Adekola *et al.,* 2017) and Indian (Arivalagan *et al.,* 2018) coconut varieties. Arivalagan *et al.* (2018) reported that the presence of a series of phenolics and flavonoids in coconut testa using a UPLC method. However, the distribution of phenolic compounds of the present study showed some dissimilarities from those previously reported elsewhere. According to those studies, a higher ferrulic acid content in methanol extract was observed when compared with caffeic, gallic, sinapic, vanillic and chlorogenic acids. Moreover, kaempferol was detected in the methanol extract in higher amounts than epichatechin, quercetin, epigallocatechin and rutin. These variations in results could be due to differences in varietal types, climatic conditions and geographical origin. Apart from this, sampling of the present study was done from coconut testa residues remained after oil extraction while those reported from other parts of the world were mainly from whole coconut testa. More importantly, this was the first attempt to investigate the inter-varietal differences in phenolic distribution among local coconut cultivars.

Evaluation of Antioxidant Properties

DPPH Radical Scavenging Activity

The IC_{50} values for DPPH radical scavenging activity of CTF are shown in Table 03. Antioxidant activity of a substance is defined as the ability to prevent or lower the oxidative damage to target molecules by combating free radicals (Mahdi-Pour *et al.,* 2012). Nowadays, investigations of plant-based antioxidant from food sources have drawn wider attention (Kedare and Singh, 2011). The radical scavenging activity of MeOH extracts of all five cultivars were remarkably higher than those of EtOAc and hexane extracts. This could be due to the high content of TPC in the MeOH extracts (Table 01). The lowest and the highest IC_{50} values for MeOH extracts were found in GT (45.37 ± 1.94 ppm) and COM (407.86 ± 23.23 ppm), respectively. The values can be aligned as GT< TT< SR< RT< COM in the ascending order. However, the IC50 values of GT, TT and SR did not show any significant ($p > 0.05$) difference while COM was significantly ($p < 0.05$) higher than the rest of the cultivars. Both EtOAc and hexane extracts of some cultivars had displayed only slight antioxidant activities. Among EtOAc extracts, RT displayed the highest antioxidant activity compared to any other cultivars while in the case of hexane extracts, both RT and SR showed weak antioxidant activity. The antioxidant activities of non-polar solvent fractions could be attributed to some of the non-phenolic antioxidants present (Ojha *et al.,* 2019). As it was noticed from Table 03, the antioxidant activities of all cultivars were significantly ($p < 0.05$) lower than the activity exhibited by ascorbic acid (IC50, 8.37 ± 0.06 ppm) and BHA (IC_{50},7.6 ± 0.03 ppm). The results further showed that the radical scavenging activity of CTF might be influenced not only by the cultivar difference but also by extracting solvents.

Ferric Reducing Power

Data presented in Table 03 shows the FRAP values corresponding to crude extracts of CTF of different cultivars. FRAP assay measures the ability of a substance to reduce ferric ion (Fe^{3+}) to ferrous (Fe^{2+}) which forms a blue complex (Appaiah *et al.,* 2016). Although some studies reported the FRAP values of coconut testa (Adekola *et al.)*, this is the first study to report the FRAP values of the partially defatted CTF of different coconut cultivars grown in Sri Lanka. Significantly ($p < 0.05$) higher FRAP values were recorded for MeOH extracts while significantly ($p < 0.05$) lower values were displayed by both hexane and EtOAc extracts. In this study, FRAP values of MeOH extracts can be aligned in the ascending order of COM< RT< SR< TT< GT and were significantly ($p < 0.05$) different among the cultivars. While the highest FRAP value was displayed for GT (1097.23 ± 1.24 µmol $FeSO_4$/g

of crude extract), the lowest value was observed for COM (78.97 ± 5.56 µmol $FeSO_4$/g of crude extract). Among the EtOAc extracts, the FRAP values of different cultivars tend to follow the order of SR< COM< GT< TT where only SR showed a significant ($p < 0.05$) difference. In this extract the highest and the lowest FRAP values were exhibited by TT (27.02 ± 0.50 µmol $FeSO_4$/g of crude extract) and RT respectively. In the case of hexane extracts, the highest and the lowest FRAP values were displayed by SR (34.95 ± 0.65 µmol $FeSO_4$/g of crude extract) and COM (4.76 ± 0.09 µmol $FeSO_4$/g of crude extract), respectively. TT also showed a higher FRAP value which was not significantly ($p > 0.05$) different from SR. However, the FRAP values of hexane extract followed the order of COM< GT< RT< TT< SR. The FRAP values of all cultivars from each solvent extract were significantly ($p < 0.05$) lower than those of ascorbic acid (10182.64 ± 28.94 µmol $FeSO_4$/g of crude extract) and BHA (9993.66 ± 8.77 50 µmol $FeSO_4$/g of crude extract). Previously, Arivalagan *et al*. (2018) noted that the FRAP values of plant materials are dependant on the effect of extracting solvents. The results of the present study further confirmed this.

Enzyme Inhibitory Activity

Pancreatic Alpha-amy and Alpha-glu are recognized as important digestive enzymes in human body and are responsible for rapid rise in postprandial blood glucose levels (Tundis *et al.,* 2010). Hence, the partial inhibition of them can be helpful in the management of postprandial rise in blood glucose levels of type-II diabetic patients (Tundis *et al.*, 2010; Bhutkar and Bhise, 2012).

The results of Alpha-amy inhibitory assay of CTF crude extracts are expressed as IC_{50} values in Table 04. Strong Alpha-amy inhibitory activities were only found among MeOH extracts of all cultivars while weak activities were observed among all extracts of hexane and some extracts of EtOAc. Further, dose dependent increment of the inhibition activities was not detected for both hexane and EtOAc extracts. According to Table 04, the IC_{50} values of MeOH extracts were found to vary from 87.35 ± 2.65 ppm to 3994.94 ± 3.60 ppm, where the lowest (highest inhibitory activity) and the highest values (lowest inhibitory activity) were displayed by TT and COM, respectively. Hence, the IC_{50} values of the extracts follow the order of TT< SR< GT< RT< COM. Although no significant ($p > 0.05$) differences were found between TT and SR, they exhibited lower IC_{50}, claiming to have a strong inhibitory effect. The IC50 value of acarbose for Alpha-amy inhibition was 82.92 ± 2.80 ppm and the value was significantly ($p < 0.05$) lower than those of COM, GT and RT.

The results of Alpha-glu inhibitory assay are depicted in Table 04. All crude extracts of different cultivars exhibited inhibitory activity against Alpha-glu. The IC_{50} values of hexane extracts varied from 8.38 ± 0.52 ppm to 65.91 ± 3.92 ppm and followed the order of GT< COM< RT< SR< TT. Further, the values of the cultivars were significantly ($p < 0.05$) different except those of SR and TT. Referring to the EtOAc extract, TT resulted in the lowest IC_{50} value which is 7.82 ± 0.40 ppm and COM resulted in the significantly ($p < 0.05$) highest value of 56.64 ± 3.37 ppm. The values of IC_{50} tended to follow the ascending order of TT< GT< RT< SR< COM, but the values showed no significant ($p > 0.05$) difference among TT, GT, RT and SR. The IC_{50} values of MeOH extracts varied from 22.53 ± 0.26 ppm to 403.32 ± 17.24 ppm following the order of GT< SR< TT< RT< COM. In this case, the IC_{50} value of COM only was significantly ($p < 0.05$) different from rest of the cultivars. In the case of acarbose, IC_{50} value obtained for Alpha-glu inhibition was 51.98 ± 2.05 ppm. The value was significantly higher ($p < 0.05$) than those of the hexane extract of COM, GT, RT, EtOAc extract of GT, RT, SR, TT and MeOH extracts of GT, SR, TT.

Table 03: Antioxidant activities of hexane, ethyl acetate and methanol extracts obtained from CTF of selected cultivars

Assay	Cultivar	Type of Extract		
		Hexane	EtOAc	MeOH
DPPH radical scavenging activity (IC_{50} value/ppm)	COM	>4000	>4000	407.86 ±23.23^{c}
	GT	>4000	>4000	45.37 ± 1.94^{a}
	RT	wk	1964.57 ± 39.32	96.85 ± 1.87^{b}
	SR	wk	>4000	55.34 ± 2.30^{a}
	TT	2077.68 ± 36.27	>4000	47.08 ± 2.25^{a}
Ferric reducing antioxidant power (µmol $FeSO_4$/g of crude extract)	COM	4.76 ± 0.09 $^{a(A)}$	22.31 ± 0.93 $^{b(B)}$	78.97 ± 5.56 $^{a(C)}$
	GT	22.68 ± 1.33 $^{b(A)}$	24.55 ± 2.99 $^{b(A)}$	1097.23 ± 1.24 $^{e(B)}$
	RT	24.67 ± 3.60 $^{b(A)}$	nd	390.69 ± 8.66 $^{b(B)}$
	SR	34.95 ± 0.65 $^{c(A)}$	9.14 ± 0.45 $^{a(A)}$	561.24 ± 26.91$^{c(B)}$
	TT	32.68 ± 2.98 $^{c(A)}$	27.02 ± 0.50 b$^{(A)}$	832.78 ± 32.66 $^{d(B)}$

Each value in the Table 0represents the mean of three replicates. In each assay, the means that do not share a similar simple superscription letter within the columns and capital superscription letter within the rows are significantly different at 95 % confident (α=0.05). Abbreviations: COM, commercial hybrid; GT, Gon Thembili; RT, Ran Thembili; SR, San Ramon; TT, TallxTall; nd, not detected; wk, weak activity: IC50, half maximal inhibitory concentration.

Table 04: Alpha amylase and alpha glucosidase inhibitory activity of hexane, ethyl acetate and methanol extracts obtained from CTF of selected cultivars

Assay	Cultivar	Type of Extract		
		Hexane	EtOAc	MeOH
Inhibition of α-amylase enzyme activity (IC_{50} value/ppm)	COM	wk	nd	3994.94 ± 3.60 ^{d}
	GT	wk	nd	143.38 ± 10.43 ^{b}
	RT	wk	wk	599.57 ± 12.41 ^{c}
	SR	wk	wk	87.35 ± 2.65 ^{a}
	TT	wk	wk	80.09 ± 4.67 ^{a}
Inhibition of α-glucosidase enzyme activity (IC_{50} value/ppm)	COM	18.84 ± 0.41 $^{b(A)}$	56.64 ± 3.37 $^{b(A)}$	403.32 ± 17.24 $^{b(B)}$
	GT	8.38 ± 0.52 $^{a(A)}$	8.56 ± 0.30 $^{a(A)}$	22.53 ± 0.26 $^{a(B)}$
	RT	40.12 ± 1.76 $^{c(B)}$	9.78 ± 0.73 $^{a(A)}$	70.43 ± 6.31 $^{a(C)}$
	SR	61.18 ± 2.21 $^{d(C)}$	12.58 ± 0.37 $^{a(A)}$	25.39 ± 1.05 $^{a(B)}$
	TT	65.91 ± 3.92 $^{d(C)}$	7.82 ± 0.40 $^{a(A)}$	36.51 ± 0.84 $^{a(B)}$

Each value in the Table 0represents the mean of three replicates. In each assay, the means that do not share a similar simple superscription letter within the columns and capital superscription letter within the rows are significantly different at 95 % confident (α=0.05). Abbreviations: COM, commercial hybrid; GT, Gon Thembili; RT, Ran Thembili; SR, San Ramon; TT, TallxTall; wk, weak activity; nd, not detected; IC50, half maximal inhibitory concentration.

Table 05: Pearson's linear correlation coefficients (R) within TPC, TFC, DPPH, FRAP, inhibition of α-amylase, inhibition of α-glucosidase and cytotoxicity of MeOH extract

	TPC	TFC	IC_{50} of DPPH	FRAP value	IC_{50} of α-Amylase	IC_{50} of α-Glucosidase
TPC	-	-	-	-	-	-
TFC	0.962	-	-	-	-	-
IC_{50} of DPPH	-0.907	-0.855	-	-	-	-
FRAP value	0.922	0.977	-0.795	-	-	-
IC_{50} of α-Amylase	-0.895	-0.837	0.975	-0.774	-	-
IC_{50} of α-Glucosidase	-0.881	-0.825	0.980	-0.770	0.996	-

Abbreviations: TPC, total phenolic content; TFC, total flavonoid content; DPPH radical scavenging activity; FRAP, ferric reducing power. Each value given in the Table 0are significant ($p \leq 0.05$).

Different cultivars exhibiting different IC50 values in different crude extracts resulted in varied potential enzyme inhibitory effect. Overall results suggest that, the inhibition of Alpha-glu activity was found to be much stronger than the inhibition for Apha-amy activity in all extract types. This finding is in agreement with the results of Adekola *et al.* (2017) who stated that the inhibitory action of coconut testa (70 % ethanol extract), towards Alpha-amy enzyme was much lower than the inhibitory action towards Alpha-glu. Factors like, the types of bioactive compounds, the mode of inhibition and affinity to the binding site of the enzyme could be responsible for this difference in inhibitory action (Marikkar *et al.,* 2016). The strong inhibition activity against of Alpha-glu and weak activity of Alpha-amy of hexane and EtOAc extracts of CTF could be attributed to some non-phenolic compounds in them. Further, Appaiah *et al.* (2014) stated that the oil fractions of coconut testa were reported to have some phytosterols and tocopherols which are mainly soluble into non-polar solvents like hexane.

Correlation Analysis of Bioactivities

Pearson's correlation coefficients (R) for bioactivities are shown in Table 05. According to the results, the bioactivities of all crude extracts exhibited strong correlations with both TPC and TFC values. Particularly, TPC and TFC of MeOH extracts showed strong positive correlations with FRAP value while showing strong negative correlations with IC_{50} values of DPPH, Alpha-amy and Alpha-glu inhibition. This data further showed that IC_{50} of DPPH have a negative correlation with FRAP values, while strong positive correlations with IC_{50} values of both Alpha-amy and Alpha-glu inhibition. The FRAP values also showed strong negative correlation with IC_{50} values of both Alpha-amy and Alpha-glu. However, these results explained the positive correlation between antioxidant activities and carbohydrate digestive enzyme inhibitory activity. These findings are compatible with the previous studies where DPPH and FRAP antioxidant activities exhibited linear correlations with inhibitory activities of Alpha-amy and Alpha-glu of some medicinal plant extracts (Sekhon-Loodu and Rupasinghe, 2019).

CONCLUSIONS

In this study, bioactivities of different solvent extracts of CTF obtained from different coconut cultivars of Sri Lanka were compared. The overall findings of this study suggest that CTF of all local coconut cultivars could be rich sources of phenolics and flavonoids and had the ability to act as a potential antioxidant and anti-hyperglycemic agents. The results further confirmed that the bioactivities of extracts of CTF varied according to the polarity of the extracting solvent. Out of the three selected extracts, MeOH extracts of all cultivars contained the highest TPC, TFC and antioxidant capacities. Among the MeOH extracts of cultivars, GT was found to display the highest TPC, TFC and antioxidant capacities. According to phytochemical analysis, several polyphenolic compounds were found in the CTF of local coconut cultivars. Out of the different polyphenolics, chlorogenic acid was predominantly presented among the cultivars investigated. This study further showed that, CTF

is a potential inhibitory source of carbohydrate hydrolyzing enzymes. These findings would therefore, emphasize the importance of CTF as an emerging source of functional food with potential health benefits to chronic ailments such as diabetes.

Conflicts of Interest

The authors declare that there is no conflict of interest.

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