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LC-ESI-QTOF-MS/MS Profiling and Antioxidant Activity of Phenolics from Custard Apple Fruit and By-Products

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Abstract: Custard apple is an edible fruit grown in tropical and subtropical regions. Due to its abundant nutrient content and perceived health benefits, it is a popular food for consumption and is utilized as a medicinal aid. Although some published research had provided the phenolic compound of custard apple, the comprehensive phenolic profiling of Australian grown custard apple is limited. Hence, this research aimed to evaluate the phenolic content and antioxidant potential by various phenolic content and antioxidant assays, followed by characterization and quantification of the phenolic profile using LC-ESI-QTOF-MS/MS and HPLC-PDA. African Pride peel had the highest value in TPC (61.69 ± 1.48 mg GAE/g), TFC (0.42 ± 0.01 mg QE/g) and TTC (43.25 ± 6.70 mg CE/g), followed by Pink's Mammoth peel (19.37 ± 1.48 mg GAE/g for TPC, 0.27 ± 0.03 mg QE/g for TFC and 10.25 ± 1.13 mg CE/g for TTC). African Pride peel also exhibited the highest antioxidant potential for TAC (43.41 ± 1.66 mg AAE/g), FRAP (3.60 ± 0.14 mg AAE/g) and ABTS (127.67 ± 4.60 mg AAE/g), whereas Pink's Mammoth peel had the highest DPPH (16.09 ± 0.34 mg AAE/g), RPA (5.32 ± 0.14 mg AAE/g), •OH-RSA (1.23 ± 0.25 mg AAE/g) and FICA (3.17 ± 0.18 mg EDTA/g). LC-ESI-QTOF-MS/MS experiment successfully characterized 85 phenolic compounds in total, encompassing phenolic acids (20), flavonoids (42), stilbenes (4), lignans (6) and other polyphenols (13) in all three parts (pulp, peel and seeds) of custard apple. The phenolic compounds in different portions of custard apples were quantified by HPLC-PDA, and it was shown that African Pride peel had higher concentrations of the most abundant phenolics. This is the first study to provide the comprehensive phenolic profile of Australian grown custard apples, and the results highlight that each part of custard apple can be a rich source of phenolics for the utilization of custard apple fruit and waste in the food, animal feeding and nutraceutical industries.

Keywords: custard apple; phenolic compounds; antioxidant potential; LC-ESI-QTOF-MS/MS; HPLC-PDA

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1. Introduction

Custard apples are popular commercial fruits widely grown in tropical and subtropical areas [1]. These fruits offer pleasant flavor and creamy taste and have high nutritional values. In Australia, the annual production of fresh custard apple is 3000 tons, and the soft edible pulp portion of the fruits is used to make different food products such as jams, candies and drinks [2,3]. Soursop (*Annona muricata*) custard apple, also called 'graviola' and 'guanabana', is a traditional custard apple fruit mainly grown for its edible and medicinal purposes. Soursop is used in commercial food products, including juice, candies and sherbets [4]. African Pride (*Annona atemoya cv.*) and Pink's Mammoth (*Annona atemoya cv.*)

are two other popular custard apple cultivars. African Pride variety is rich in phytochemicals (catechin and epicatechin gallate) and phytonutrients, including carbohydrates and crude proteins, whereas Pink's Mammoth has a high mineral content, including calcium and zinc [5].

Phytochemicals present in custard apples recently gained substantial interest, particularly for the investigation of phenolic compounds [6]. Polyphenols are secondary metabolites abundantly present in different plants, including fruits, vegetables and medicinal plants. They can be classified into five major classes, namely phenolic acids, flavonoids, lignans, stilbenes and phenol alcohols based on the structure [7]. Polyphenols can act as antioxidants that scavenge free radicals directly or indirectly via various mechanisms. The free radical scavenging capacity of polyphenols is related to their structure, by donating either hydrogen atoms or electrons to free radicals and stabilizing the reactive species [8]. Phenolic content can be evaluated by various assays, including total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC), while the antioxidant capacity of plants can be effectively measured and estimated by a series of assays, including 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), ferric reducing antioxidant power (FRAP), reducing power activity assay (RPA), hydroxyl radical scavenging activity ($^{\bullet}$OH-RSA), ferrous ion chelating activity (FICA) and total antioxidant capacity (TAC) [8].

Apart from measuring the phenolic contents and antioxidant potential, the separation and characterization of individual phenolic compounds with strong antioxidant potential in custard apples are of great interest. Previously, several phenolic compounds such as catechin and epicatechin have been identified in different custard apples using high-performance liquid chromatography equipped with a photodiode array (HPLC-PDA) and LC-MS/MS techniques [1,9]. Liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-MS-QTOF-MS/MS) is a high-resolution and highly sensitive technique widely used for the screening and characterization of phenolic compounds in different plant materials, whereas HPLC-PDA allows the quantification of phenolic compounds [10,11]. Although researchers have reported some bioactive compounds from custard apple fruits, only a few studies have focused on the whole phenolic profiles of Australian grown custard apples. In addition, there is a lack of studies on waste material such as peel and seed of custard apples which have the potential to be utilized for extraction of bioactive compounds.

In the present research, the phenolic profiles of three different Australian grown custard apples (soursop, African Pride and Pink's Mammoth) were characterized using LC-ESI-QTOF-MS/MS and quantified through HPLC-PDA. Further analyses, which included the TPC, TFC and TTC measurements and DPPH, ABTS, FRAP, RPA, $^{\bullet}$OH-RSA, FICA and TAC antioxidant assays, were performed. The outcome of this study enables us to understand the phenolic composition of different Australian grown custard apples for their commercial utilization in the preparation of functional, nutraceutical and pharmaceutical products.

2. Materials and Methods

2.1. Chemicals and Reagents

Most of the chemicals used for the extraction and characterization were analytical grade and purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Folin–Ciocalteu phenol reagent, vanillin (≥97%), gallic acid (≥98%), aluminum chloride hexahydrate (≥98%), ascorbic acid (≥99%), quercetin (≥95%), catechin (≥96%), 2,2′-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-*s*-triazine (TPTZ) (≥98%), HCl (≥99%), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (≥98%) and potassium persulfate (≥99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA) for the estimation of phenolic content and a series of antioxidant measurement trials. Sodium carbonate (≥99.5%), sulfuric acid (≥95%), ethanol (≥99.5%), acetic acid (≥99%), ferric chloride (Fe(III)$Cl_3 \cdot 6H_2O$) (≥97%) and sodium acetate (≥99.5%), were purchased from Thermo Fisher (Scoresby, Melbourne, VIC, Australia). Methanol

(HPLC Grade, 99.8%), acetic acid (≥99%) and acetonitrile (≥99.9%) were purchased from Sigma-Aldrich (St. Louis, MO, USA) for HPLC. The HPLC standards, including quercetin (≥95%), protocatechuic acid (≥97%), *p*-coumaric acid (≥98.0%), catechin (≥98%), chlorogenic acid (≥95%), epicatechin (≥98%), quercetin-3-rhamnoside (≥97%), *p*-hydroxybenzoic acid (≥97%), syringic acid (≥95%) and quercetin-3-glucoside (≥95%), were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sample Preparation

Three physiologically mature Australian grown custard apples (soursop, African Pride and Pink's Mammoth) were purchased from a retail market in Melbourne, Australia. The fruits were cleaned and separated into peel, pulp and seed portions. Samples were trimmed into slices, freeze-dried at −20 °C for 48 h and lyophilized at −45 °C/50 MPa by Dynavac engineering FD3 Freeze Drier (Belmont, WA, Australia) and Edwards RV12 oil-sealed rotary vane pump (Bolton, UK) [12]. The freeze-dried peels, seeds and pulps were ground into powders and stored at −20 °C.

2.3. Extraction of Phenolic Compounds

Five grams of each sample was extracted by 15 mL of 80% ethanol [13]. All samples were homogenized with an Ultra-Turrax T25 Homogenizer for 30 s at 10,000 rpm (IKA, Staufen, Germany), followed by the incubation at 120 rpm and 4 °C for 18 h in a shaker incubator (ZWYR-240, Labwit, Ashwood, VIC, Australia). After incubation, samples were centrifuged by refrigerated Centrifuge (Hettich Rotina 380R, Tuttlingen, Germany) at 5000 rpm for 15 min. The supernatant was immediately collected and stored at −20 °C for further analysis.

2.4. Polyphenol Estimation and Antioxidant Assays

The estimation of polyphenols was performed by three assays (TPC, TFC and TTC), while the antioxidant capacity was measured by seven diverse assays (DPPH, FRAP, ABTS, RPA, •OH-RSA, FICA and TAC). All these methods except TAC were reported by Gu et al. [14], Zhu et al. [15] and Suleria et al. [16]. The data were acquired by the Multiskan Go microplate photometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.4.1. Determination of Total Phenolic Content (TPC)

The total phenolic content of the extracts was determined by the Folin–Ciocalteu method with some modifications [17]. A 25 µL aliquot of extract was mixed with Folin–Ciocalteu reagent solution and 200 µL water in a 96-well plate (Corning Inc., Midland, NC, USA). A 5 min incubation at room temperature was required before 25 µL 10% (*w*:*w*) sodium carbonate was added. A further incubation (60 min) at 25 °C in a dark room was performed, followed by the measurement of absorbance at 765 nm by a spectrophotometer plate reader. The quantification of total phenolic content was based on a standard curve generated from gallic acid with concentrations from 0 to 200 µg/mL, and the results were expressed as mg of gallic acid equivalents per gram (mg GAE/g).

2.4.2. Determination of Total Flavonoid Content (TFC)

The total flavonoid contents were estimated by the modified aluminum chloride method [18]. An 80 µL aliquot of extract was transferred to a 96-well plate and subsequently mixed with 80 µL of 2% ethanolic aluminum chloride and 120 µL of 50 g/L sodium acetate solution before being transferred to a dark room and incubated at 25 °C for 2.5 h. The quantification of total phenolic content was based on a standard curve generated from quercetin with concentrations from 0 to 50 µg/mL, and the results were expressed as mg of quercetin equivalents per gram (mg QE/g).

2.4.3. Determination of Total Tannin Content (TTC)

The total tannin contents were determined by a vanillin–sulfuric acid method with some modification [19]. Twenty-five microliters of 32% sulfuric acid was added to 25 µL of sample and 150 µL of 4% vanillin solution in a 96-well plate which was then incubated at room temperature for 15 min in a dark room. Subsequently, the measurement of absorbance was carried out at 500 nm by using the plate reader. The quantification of total tannin content was based on a standard curve generated from catechin with concentrations from 0 to 100 µg/mL, and the results were expressed as mg of catechin equivalents (CE) per gram (mg CE/g).

2.4.4. 2,2′-Diphenyl-1-Picrylhydrazyl (DPPH) Assay

The DPPH assay was adopted to examine the free radical scavenging ability, and the procedure had some modifications made to the published method [20]. A 40 µL aliquot of extract was mixed with 260 µL of DPPH ethanolic solution (0.1 mM) in a 96-well plate. Further, the plate was incubated for 30 min at 25 °C. The absorbance was measured at 517 nm, and the result was quantified by a calibration curve generated by ascorbic acid with gradient concentrations (0–50 µg) and expressed as mg of ascorbic acid equivalents per g (mg AAE/g).

2.4.5. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay measured the capacity of extracts to reduce the ferric ions to ferrous ions, and the method adopted in this study was modified according to a previous method [20]. The FRAP reagent was prepared by mixing sodium acetate solution (300 mM), 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution (10 mM) and Fe(III) solution (20 mM) in the ratio of 10:1:1. A 20 µL aliquot of extract was added to a 96-well plate and was then mixed with 280 µL of prepared FRAP reagents followed by incubation at 37 °C for 10 min. The measurement of absorbance was performed at 593 nm in a plate reader. Ascorbic acid with a series of dilutions (ranging from 0 to 50 µg/mL) was used to plot a standard curve, and the results were expressed as mg of ascorbic acid equivalents per g (mg AAE/g).

2.4.6. 2,2′-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid (ABTS) Assay

The estimation of ABTS scavenging activity was performed by modifying $ABTS^+$ radical cation decolorization assay [20]. The $ABTS^+$ radical solution was prepared by mixing 7 mmol/L of ABTS solution with 140 mM potassium persulfate solution, followed by storage in a dark room for 16 h. Further dilution of the solution with ethanol was required to give absorbance at 734 nm. A 10 µL aliquot of extract and 290 µL diluted solution were mixed in a 96-well plate and then incubated at room temperature for 6 min in the dark. Further, the measurement of absorbance was performed at 734 nm in a plate reader. The results were expressed as mg of ascorbic acid equivalent per g (mg AAE/g), and the standard curve was generated with a series of dilutions of ascorbic acid concentrations ranging from 0 to 2000 µg/mL.

2.4.7. Reducing Power Assay (RPA)

The reducing power activity was determined by modifying the method of Ferreira et al. [21]. Sequential addition of 10 µL of extract, 25 µL of 0.2 M sodium phosphate buffer (pH 6.6) and 25 µL of $K_3(Fe(CN)_6]$) was followed by incubation at 25 °C for 20 min. Then, 25 µL of 10% TCA solution was added to stop the reaction, followed by the addition of 85 µL of water and 8.5 µL of $FeCl_3$. The solution was further incubated for 15 min at 25 °C. Then, the absorbance was measured at 750 nm. Ascorbic acid from 0 to 500 µg/mL was used to obtain a standard curve, and data were presented as mg AAE/g fw.

2.4.8. Hydroxyl Radical Scavenging Activity ($^{\bullet}$OH-RSA)

The Fenton-type reaction method of Smirnoff and Cumbes [22] was used to determine $^{\bullet}$OH-RSA with some modifications. Fifty microliters of extract was mixed with 50 µL of

6 mM $FeSO_4.7H_2O$ and 50 µL of 6 mM H_2O_2 (30%), followed by incubation at 25 °C for 10 min. After incubation, 50 µL of 6 mM 3-hydroxybenzoic acid was added, and absorbance was measured at a wavelength of 510 nm. Ascorbic acid from 0 to 300 µg/mL was used to obtain a standard curve, and data were presented as mg AAE/g fw.

2.4.9. Ferrous Ion Chelating Activity (FICA)

The Fe^{2+} chelating activity of the sample was measured according to the method of Dinis et al. [23] with modifications. Fifteen microliters of extract was mixed with 85 µL of water, 50 µL of 2 mM ferrous chloride (with additional 1:15 dilution in water) and 50 µL of 5 mM ferrozine (with additional 1:6 dilution in water), followed by incubation at 25 °C for 10 min. Then, the absorbance was measured at a wavelength of 562 nm. Ethylenediaminetetraacetic acid (EDTA) in concentrations from 0 to 30 µg/mL was used to obtain a standard curve, and data were presented as mg EDTA/g fw.

2.4.10. Determination of Total Antioxidant Capacity (TAC)

The total antioxidant capacity was measured by the modified TAC assay [24]. The TAC reagent was prepared by mixing 0.6 M H_2SO_4, 28 mM Na_3PO_4 and 4 mM ammonium molybdate. The extract was mixed with the prepared dye in a 96-well plate, followed by incubation at 95 °C for 90 min. The plate was then cooled at room temperature for 10 min, and the absorbance was measured at 765 nm in a microplate photometer. The results were expressed as mg ascorbic acid equivalents per gram (mg AAE/g), and the standard curve was generated by series dilution of ascorbic acid solution ranging from 0 to 300 µg/mL.

2.5. Characterization of Phenolic Compounds through LC-ESI-QTOF-MS/MS

The characterization of phenolic compounds was carried out by LC-ESI-QTOF-MS/MS by adapting the method of Zhong et al. [25]. The process was performed on an Agilent 1200 series HPLC (Agilent Technologies, CA, USA) equipped with an Agilent 6520 Accurate-Mass Q-TOF LC-MS/MS (Agilent Technologies, CA, USA). A Synergi Hydro-RP 80 °A LC reverse-phase column with the diameter of 250 × 4.6 mm inside and particle diameter of 4 µm (Phenomenex, Torrance, CA, USA) was used for the separation of compounds. Mobile phase A was prepared with acetic acid/water (0.5:99.5, *v*/*v*), while mobile phase B was prepared with acetonitrile/water/acetic acid (50:49.5:0.5, *v*/*v*/*v*). Both mobile phases A and B were degassed under vacuum at room temperature (25 °C) for 15 min. All extracts were filtered by syringe (Kinesis, Redland, QLD, Australia) coupled with the 0.45 µm syringe filter (Thermo Fisher Scientific Inc., Waltham, MA, USA) before transferring to vials for the HPLC. The injection volume was 6 µL for each extract, and the flow rate was set at 0.8 mL/min. Elution conditions were as follows: 0 min with 10% B, 20 min with 25% B, 30 min with 35% B, 40 min with 40% B, 70 min with 55% B, 75 min with 80% B, 77 min with 100% B, 79 min with 100% B and 82–85 min with isocratic 10% B. The MS/MS peak identification was carried out in both negative and positive modes, and the mass spectra were obtained within the range of *m*/*z* 50 to 1300 amu. Further, MS/MS analyses were carried out in automatic mode with multiple collision energy (10, 15 and 30 eV) for fragmentation. The temperature of nitrogen gas was set at 300 °C, and the flow rate was set at 5 L/min. The temperature of sheath gas was set at 250 °C, and the flow rate was set at 11 L/min with atomizing gas pressure 45 psi. The capillary and nozzle voltages were set at 3.5 kV and 500 V, respectively. Data collection and subsequent analysis were conducted using Agilent LC-ESI QTOF-MS/MS Mass Hunter Qualitative Software—B.03.01 (Agilent Technologies, Santa Clara, CA, USA).

2.6. Quantification of Polyphenols via HPLC-PDA Analysis

The quantification of phenolic compounds in the various sections (peel, pulp and seed) of custard apples was carried out by using the method of Ma et al. [26]. The detection for diverse phenolic constituents was carried out under three different wavelengths (280, 320 and 370 nm). The column and conditions followed the LC-MS/MS methodology, but the in-

jection volume was 20 µL. A total of 10 targeted phenolic compounds were quantified in the present study, including 5 phenolic acids (protocatechuic acid, *p*-hydroxybenzoic acid, chlorogenic acid, syringic acid and *p*-coumaric acid) and 5 flavonoids (catechin, epicatechin, quercetin-3-glucoside, quercetin-3-rhamnoside and quercetin). Data collection and analysis were completed using Agilent LC-ESI-QTOF-MS/MS Mass Hunter Qualitative Software.

2.7. Statistical Analysis

All analyses were carried out in triplicate. The results are shown as mean ± standard deviation (SD). Tukey's test in one-way analysis of variance (ANOVA) was performed using Minitab 18 Statistical software (Minitab Inc., State College, PA, USA) for the comparison of the concentration levels between samples' estimated phenolic content and antioxidant activities. $p < 0.05$ was selected for the significance level for the Tukey's test.

3. Results and Discussion

3.1. Phenolic Content Estimation (TFC, TFC, TTC)

Custard apples, including atemoya and soursop, have been proven to have a considerable number of phenolic compounds [27,28]. The phenolic contents in this study were determined by TPC, TFC and TTC (Table 1).

In the TPC assay, the African Pride peel had the highest (61.69 ± 1.48 mg GAE/g) phenolic content, followed by Pink's Mammoth and soursop peel. With regards to seed and pulp portions, African pride had the highest phenolic content (1.40 ± 0.07 mg GAE/g and 3.81 ± 0.17 mg GAE/g, respectively) when compared to the other varieties. Previously, Manochai et al. [5] reported values that range between 33.8 and 140.4 mg GAE/g, where the African pride showed higher values than those found in our study. The variation in the concentration of total phenolics in the custard apple might be due to the different concentrations of solvents used for extraction [24]. With respect to the TPC content, the peel extracts have significantly higher levels ($p < 0.05$) of total phenolic contents than the seeds and pulps. Sasidharan and Jayadev [29] reported that the higher phenolic content in the peel might be due to the pericarp being exposed to stress directly from the environment, unlike the seed and the pulp which are enclosed.

African Pride was found to have the most abundant TFC values for peels and pulps (0.42 ± 0.01 mg GAE/g and 0.38 ± 0.01 mg GAE/g), while Pink's Mammoth exhibited the greatest value (0.21 ± 0.01 mg GAE/g) within seeds. Previously, Santos et al. [27] reported that the flavonoid content was the same as the TFC in African Pride and in Pink's Mammoth pulp.

To the best of our knowledge, only limited studies have been reported on TTC of custard apples. In the present study, the TTC was higher in peels of custard apples than in the pulp and seed. African Pride and Pink's Mammoth peel have significantly ($p < 0.05$) higher concentrations of tannins. Previously, the soursop pulp extracted with 50% methanol was found to have a higher value than that found in our study [27]. Lydia et al. [30] used various solvents (acetone, ethanol and water) for extraction and reported that the water extract had the lowest tannin content.

Overall, the African Pride peel variety of custard apple has higher total phenolics, flavonoids and tannin contents than the other two varieties, namely Pink's Mammoth and soursop, particularly in the peel and the seed.

Table 1. Estimation of phenolic content and antioxidant potential of different custard apple samples.

Assays	Peel			Seed			Pulp		
	African Pride	**Pink's Mammoth**	**Soursop**	**African Pride**	**Pink's Mammoth**	**Soursop**	**African Pride**	**Pink's Mammoth**	**Soursop**
TPC (mg GAE/g)	61.69 ± 1.48^{a}	19.37 ± 0.27^{b}	4.07 ± 0.20^{c}	1.40 ± 0.07^{a}	1.10 ± 0.04^{a}	0.39 ± 0.01^{b}	3.81 ± 0.17^{a}	0.95 ± 0.01^{b}	1.19 ± 0.06^{b}
TFC (mg QE/g)	0.42 ± 0.01^{a}	0.27 ± 0.03^{b}	0.06 ± 0.01^{c}	0.09 ± 0.01^{b}	0.21 ± 0.01^{a}	0.09 ± 0.01^{b}	0.38 ± 0.01^{a}	0.06 ± 0.01^{b}	0.04 ± 0.01^{b}
TTC (mg CE/g)	43.25 ± 6.70^{a}	10.25 ± 1.13^{b}	0.37 ± 0.01^{c}	0.45 ± 0.01^{a}	-	0.10 ± 0.01^{b}	1.35 ± 0.06^{a}	0.17 ± 0.01^{b}	0.04 ± 0.01^{c}
TAC (mg AAE/g)	43.41 ± 1.66^{a}	10.43 ± 0.20^{b}	0.83 ± 0.01^{c}	2.48 ± 0.05^{b}	2.87 ± 0.07^{a}	1.14 ± 0.01^{c}	1.33 ± 0.04^{a}	0.88 ± 0.02^{b}	0.32 ± 0.01^{c}
DPPH (mg AAE/g)	1.87 ± 0.09^{b}	16.09 ± 0.34^{a}	0.70 ± 0.01^{c}	1.39 ± 0.08^{a}	0.68 ± 0.02^{b}	0.42 ± 0.01^{c}	6.76 ± 0.12^{b}	13.75 ± 0.67^{a}	0.03 ± 0.01^{c}
FRAP (mg AAE/g)	3.60 ± 0.14^{a}	0.43 ± 0.01^{b}	0.11 ± 0.01^{b}	0.34 ± 0.01^{a}	0.03 ± 0.01^{c}	0.14 ± 0.01^{b}	0.06 ± 0.01^{a}	0.07 ± 0.01^{a}	0.02 ± 0.01^{a}
ABTS (mg AAE/g)	127.67 ± 4.60^{a}	33.40 ± 0.93^{b}	1.08 ± 0.01^{c}	2.22 ± 0.14^{a}	1.21 ± 0.01^{b}	0.73 ± 0.04^{c}	7.02 ± 0.15^{a}	1.86 ± 0.01^{b}	0.49 ± 0.01^{c}
RPA (mg AAE/g)	4.75 ± 1.2^{b}	5.32 ± 0.14^{a}	1.73 ± 0.43^{c}	5.63 ± 0.24^{a}	4.96 ± 0.01^{b}	1.24 ± 0.12^{c}	6.47 ± 0.03	3.15 ± 0.09	1.45 ± 0.03
$^{\bullet}$OH-RSA (mg AAE/g)	0.94 ± 0.31^{b}	1.23 ± 0.25^{a}	0.93 ± 0.39^{b}	0.42 ± 0.15^{b}	0.15 ± 0.09^{c}	0.79 ± 0.07^{a}	0.18 ± 0.04^{b}	1.14 ± 0.04^{a}	0.97 ± 0.07^{a}
FICA (mg EDTA/g)	1.11 ± 0.43^{b}	3.17 ± 0.18^{a}	0.75 ± 0.21^{c}	2.14 ± 0.14^{a}	1.58 ± 0.12^{b}	2.17 ± 0.19^{a}	0.98 ± 0.09^{a}	0.13 ± 0.01^{b}	1.28 ± 0.01^{a}

The result is displayed as mean ± standard deviation ($n = 3$); $^{a–d}$ indicates that means in a row are different at the significant level of ($p < 0.05$) (within peel, seed and pulp) using one-way analysis of variance (ANOVA) and Tukey's test. GAE: gallic acid equivalents; QE: quercetin equivalents; CE: catechin equivalents; AAE: ascorbic acid equivalents; EDTA: ethylenediaminetetraacetic acid; TPC: total phenolic content; TFC: total flavonoid content; TTC: total tannin content; DPPH: 2,2′-diphenyl-1-picrylhydrazyl; FRAP: ferric reducing antioxidant power, ABTS: 2,2′-azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid; RPA: reducing power assay; $^{\bullet}$OH-RSA: hydroxyl radical scavenging activity; FICA: ferrous ion chelating activity; TAC: total antioxidant content.

3.2. Antioxidant Activity (FRAP, DPPH, ABTS, RPA, •OH-RSA, FICA and TAC)

In our current study, the antioxidant potential was estimated by FRAP, DPPH, ABTS, •OH-RSA, FICA, RPA and TAC assays, and the results are shown in Table 1.

In FRAP assay, the electron transfer method was used to measure the capacity of reducing Fe^{3+} to Fe^{2+} [31]. The African Pride peel (3.60 ± 0.14 mg AAE/g) and seed (0.34 ± 0.01 mg AAE/g) had significantly higher antioxidant potential than the other two varieties, whereas no significant difference was observed within the pulps. Akomolafe and Ajayi [32] showed that the peel of soursop exhibited higher antioxidant potential than the pulp extract, which indicates that the peel extract has the ability to deactivate the initiation of lipid peroxidation in tissues.

In DPPH assay, the free radical scavenging activity was determined, which is most likely attributed to the phenolic compounds [33]. In our current study, Pink's Mammoth peel (16.09 ± 0.34 mg AAE/g) and pulp (13.75 ± 0.67 mg AAE/g) had higher antioxidant activity than African pride and soursop varieties. However, in the seed comparison, the African Pride had higher antioxidant potential. Previous studies also demonstrated that high antioxidant activity was exhibited in different portions of soursop grown in Panama [34,35]. In the ABTS assay, the antiradical scavenging activities are determined based on the hydrogen atom donating tendency of polyphenols [33]. Our present study showed that African Pride variety had a significantly higher ($p < 0.05$) value than Pink's Mammoth and soursop. Previously, Agu and Okolie [36] reported that the antioxidant activity might be due to the presence of phenolic compounds and acetogenins, including 15-acetyl guanacone, in soursop, and such compounds are abundant in annonaceous fruit.

In RPA, •OH-RSA and FICA assays, Pink's Mammoth peel had higher antioxidant potential than other varieties. In RPA, African Pride seed had the highest antioxidant potential, followed by pink mammoth and soursop. In •OH-RSA and FICA assays, soursop seed had high antioxidant activity compared to other varieties. African Pride, Pink's Mammoth and soursop pulps showed highly significant antioxidant activity in RPA, •OH-RSA and FICA assays. Previously, it was found that the reducing power of *A. squamosa* leaf extract is directly proportional to the concentration of the extract and ranged from 0.984 to 0.91 mg/mL [37]. Previously, Nandhakumar and Indumathi [38] showed that the •OH scavenging activity was present in custard apple pulp and the range in aqueous extract was 13.27–74.65%. Our study shows results similar to those of the previous studies. To our best knowledge, this is the first time that custard apple's antioxidant potential was analyzed through the FICA assay.

In the TAC assay, which was conducted by reducing molybdenum(VI) to molybdenum(V) in the presence of phenolics, the peels of the custard apple varieties had generally higher values than pulps and seeds. The highest value was observed in African Pride peel (43.41 ± 1.66 mg GAE/g), followed by Pink's Mammoth peel (10.43 ± 0.20 mg AAE/g) and Pink's Mammoth seed (2.87 ± 0.07 mg AAE/g).

Overall, the African Pride variety exhibited higher antioxidant potential when compared with other varieties. Besides, the peels had higher antioxidant potential than the seeds and pulps. Serquiz et al. [24] reported that the peels and seeds of the custard apples, which constitute 40% of the fruit, go into food wastage and account for 10 tons of the inedible parts of the fruit discarded. Based on our current research, the inedible parts of the fruit can be utilized in the field of biotechnology and pharmacology [24,32].

3.3. LC-ESI-QTOF-MS/MS Characterization

The qualitative analysis of phenolic compounds from ethanolic extracts of custard apples was carried out by LC-ESI-QTOF-MS/MS. Phenolic compounds in nine custard apple samples were identified based on the retention time (RT), m/z value and MS/MS spectra in both negative and positive ionization modes ($[M - H]^-$/$[M + H]^+$) through Agilent LC-ESI-QTOF-MS/MS Mass Hunter Qualitative Software and Personal Compound Database and Library (PCDL). The library score (higher than 80) and mass error (less than 5 ppm) were chosen as the main criteria for the selection of compounds for further MS/MS analysis and m/z characterization (Supplementary Materials, Figures S1–S3). In the present study, a total of 85 phenolic compounds were tentatively identified via LC-MS/MS,

including 20 phenolic acids, 42 flavonoids, 4 stilbenes, 6 lignans and 13 other polyphenols, as shown in Table 2.

3.3.1. Phenolic Acids

There were four subclasses of phenolic acids identified in custard apple samples, namely hydroxybenzoic acid, hydroxycinnamic acids, hydroxyphenylacetic acids and hydroxyphenylpropanoic acids.

Hydroxybenzoic Acid and Hydroxycinnamic Acid Derivatives

Three hydroxybenzoic acids were detected in three out of nine custard apple samples. Compound **3** with $[M - H]^-$ ion at m/z 299.0778 was only detected in African Pride seed and was tentatively characterized as 4-hydroxybenzoic acid 4-*O*-glucoside. The product ions at m/z 255 and m/z 137 from precursor ion correspond to the loss of CO_2 (44 Da) and hexosyl moiety (162 Da), respectively [39]. Rosmarinic acid is a typical hydroxycinnamic derivative exclusively detected in African Pride peel, yielding the product ion at m/z 179, indicating the presence of caffeic acid ion. This compound was previously found in rosemary, basil and other Lamiaceae spices, as reported by Hossain et al. [40]. Compound **16** ($[M - H]^-$ ion at m/z 179.0349) was tentatively characterized as caffeic acid, and the compound produced fragments at m/z 143 ($[M - H - 36]^-$, loss of two water molecules) and m/z 133 ($[M - H - 46]^-$, loss of HCOOH group), conforming to the fragmentation pattern of caffeic acid [41]. In a previous study, caffeic acid was likewise identified in the peel and seed of *A. crassiflora* in the research of Roesler et al. [42]. Through HPLC-DAD analysis, Nam et al. [43] quantified the concentration of caffeic acid in the tissues derived from another fruit from the Annonaceae family called pawpaw (*Asimina triloba*), and the value was around 21.23 mg/100g in 80% methanol. 3-Sinapoylquinic acid ($[M - H]^-$ ion at m/z 397.1143) was proposed for compound **6** detected in the pulp of African Pride and Pink's Mammoth, yielding fragment ions at m/z 223 (sinapic acid ion) and m/z 179 (sinapic acid − COO) [44]. Compound **17** ($[M - H]^-$ at m/z 326.1046) was characterized as *p*-coumaroyl tyrosine based on the comparison with previous study as the product ion was produced at m/z 282, corresponding to the loss of 44 Da (a carbon dioxide molecule) from the precursor ion [45].

3.3.2. Flavonoids

Flavonoids are another important class of phenolic compounds and have an essential role in biological activities in plants. Based on the MS spectrum and data comparison with the literature, flavonoids accounted for nearly half of the total compounds that were identified and characterized, including the subclasses of anthocyanins, dihydrochalcones, dihydroflavonols, flavanols, flavanones, flavones, flavonols and isoflavonoids.

Anthocyanin, Dihydroflavonol and Flavonol Derivatives

Anthocyanidins, including cyanidin, pelargonidin, delphinidin, malvidin, petunidin and peonidin, are found in fruits and vegetables, and anthocyanins are the derivatives of anthocyanidins [46]. Compounds **24** and **25** were detected in both modes of ionization with precursor ions at m/z 466.1116 and m/z 612.1694, respectively, and were tentatively identified as delphinidin 3-*O*-glucoside and cyanidin 3,5-*O*-diglucoside. Delphinidin 3-*O*-glucoside had product ion at m/z 303 due to the loss of glucose, whereas cyanidin 3,5-*O*-diglucoside yielded MS^2 ions at m/z 449 and m/z 287, corresponding to the loss of $C_6H_{10}O_5$ and $2C_6H_{10}O_5$ [47,48]. Petunidin 3-*O*-(6''-acetyl-glucoside) (Compound **26** with $[M + H]^+$ at m/z 522.1373) was detected in the peel of soursop with product ion at m/z 317, indicating the loss of 204 Da (acetyl and glucose moiety) from the precursor ion [47]. In addition, three other anthocyanidin derivatives were characterized as two cyanidin glucoside derivatives (Compounds **21** and **22**) and one isopeonidin derivative (Compound **23**) in the present research.

Table 2. Characterization of phenolic compounds in custard apples by using LC-ESI-QTOF-MS/MS.

No.	Proposed Compounds	Molecular Formula	RT (min)	Ionization (ESI^+/ESI^-)	Molecular Weight	Theoretical (*m*/*z*)	Precursor (*m*/*z*)	Error (ppm)	MS/MS Product Ions	Samples
				Phenolic acid						
				Hydroxybenzoic acids						
1	4-Hydroxybenzoic acid 4-*O*-glucoside	$C_{13}H_{16}O_8$	14.564	$[M-H]^-$	300.0845	299.0772	299.0778	2.01	255, 137	ASE
2	3,4-*O*-Dimethylgallic acid	$C_9H_{10}O_5$	16.475	** $[M+H]^+$	198.0528	199.0601	199.0605	2.01	153, 139, 125, 111	ASE, * PPE
3	Gallic acid 4-*O*-glucoside	$C_{13}H_{16}O_{10}$	84.878	$[M-H]^-$	332.0740	331.0670	331.0660	−2.42	169, 125	SSE
				Hydroxycinnamic acids						
4	Rosmarinic acid	$C_{18}H_{16}O_8$	7.130	$[M-H]^-$	360.0845	359.0772	359.0788	4.46	197, 179, 161	APE
5	3-*p*-Coumaroylquinic acid	$C_{16}H_{18}O_8$	13.656	** $[M-H]^-$	338.1002	337.0929	337.0941	3.56	265, 173, 162, 127	APE, * PSE
6	3-Sinapoylquinic acid	$C_{18}H_{22}O_{10}$	14.204	** $[M-H]^-$	398.1213	397.1140	397.1143	0.76	223, 179	* APE, APU, PPU
7	Caffeic acid 3-*O*-glucuronide	$C_{15}H_{16}O_{10}$	14.339	** $[M-H]^-$	356.0743	355.0670	355.0660	−2.80	179	APE, * PPE
8	Sinapic acid	$C_{11}H_{12}O_5$	17.036	** $[M-H]^-$	224.0685	223.0612	223.0602	−4.50	205, 179, 163	ASE, * PSE
9	Ferulic acid 4-*O*-glucuronide	$C_{16}H_{18}O_{10}$	18.378	** $[M-H]^-$	370.0900	369.0827	369.0844	4.61	193, 178	* APE, ASE
10	Cinnamic acid	$C_9H_8O_2$	20.992	** $[M-H]^-$	148.0524	147.0451	147.0456	3.40	103	* APE, APU, ASE, PPE, PSE
11	m-Coumaric acid	$C_9H_8O_3$	23.994	** $[M-H]^-$	164.0473	163.0400	163.0406	3.68	195, 177, 145, 117	* APE, PPE
12	1,5-Dicaffeoylquinic acid	$C_{25}H_{24}O_{12}$	24.236	** $[M-H]^-$	516.1268	515.1195	515.1176	−3.70	353, 335, 191, 179	APE, PPE, * PSE
13	3-Caffeoylquinic acid	$C_{16}H_{18}O_9$	24.856	** $[M-H]^-$	354.0951	353.0878	353.0867	−3.10	253, 190, 144	APE, ASE, PPE, * SPE
14	3-Feruloylquinic acid	$C_{17}H_{20}O_9$	25.237	** $[M-H]^-$	368.1107	367.1034	367.1045	3.00	298, 288, 192, 191	APE, * PPE, PSE
15	*p*-Coumaric acid 4-*O*-glucoside	$C_{15}H_{18}O_8$	25.266	$[M-H]^-$	326.1002	325.0929	325.0935	1.85	169	APE, * ASE
16	Caffeic acid	$C_9H_8O_4$	31.284	$[M-H]^-$	180.0423	179.0350	179.0349	−0.56	151, 143, 133	APE
17	*p*-Coumaroyl tyrosine	$C_{18}H_{17}NO_5$	44.401	** [M − H]-	327.1107	326.1034	326.1046	3.68	282	* ASE, PPE, PSE
				Hydroxyphenylacetic acids						
18	3,4-Dihydroxyphenylacetic acid	$C_8H_8O_4$	31.930	** $[M-H]^-$	168.0423	167.0350	167.0353	1.80	149, 123	* APE, ASE, PPE, PSE
19	2-Hydroxy-2-phenylacetic acid	$C_8H_8O_3$	36.283	** $[M-H]^-$	152.0473	151.0400	151.0404	2.65	125	APE, * ASE, PPE
				Hydroxyphenylpropanoic acids						
20	Dihydroferulic acid 4-*O*-glucuronide	$C_{16}H_{20}O_{10}$	23.531	$[M-H]^-$	372.1056	371.0983	371.0980	−0.81	175	APE
				Flavonoids						
				Anthocyanins						
21	Cyanidin 3-*O*-(6″-p-coumaroyl-glucoside)	$C_{30}H_{27}O_{13}$	16.724	$[M+H]^+$	595.1452	596.1525	596.1554	4.86	287	* PPE, PPU
22	Cyanidin 3-*O*-diglucoside-5-*O*-glucoside	$C_{33}H_{41}O_{21}$	27.059	$[M+H]^+$	773.2140	774.2213	774.2200	−1.68	610, 464	PPE

Table 2. *Cont.*

No.	Proposed Compounds	Molecular Formula	RT (min)	Ionization (ESI^+/ESI^-)	Molecular Weight	Theoretical (*m*/*z*)	Precursor (*m*/*z*)	Error (ppm)	MS/MS Product Ions	Samples
23	Isopeonidin 3-*O*-arabinoside	$C_{21}H_{21}O_{10}$	30.284	** $[M + H]^+$	433.1135	434.1208	434.1209	0.23	271, 253, 243	PPE, * SPE
24	Delphinidin 3-*O*-glucoside	$C_{21}H_{21}O_{12}$	42.731	** $[M + H]^+$	465.1033	466.1106	466.1116	2.15	303	* APE, PPE, PSE
25	Cyanidin 3,5-*O*-diglucoside	$C_{27}H_{31}O_{16}$	42.927	** $[M + H]^+$	611.1612	612.1685	612.1694	1.47	449, 287	APE, ASE, * PPE
26	Petunidin 3-*O*-(6″-acetyl-glucoside)	$C_{24}H_{25}O_{13}$	59.402	$[M + H]^+$	521.1295	522.1368	522.1373	0.96	317	SPE
					Dihydrochalcones					
27	Phloridzin	$C_{21}H_{24}O_{10}$	17.951	** $[M - H]^-$	436.1369	435.1296	435.1289	−1.61	273	APE, PPE, *PPU
28	3-Hydroxyphloretin 2′-*O*-glucoside	$C_{21}H_{24}O_{11}$	22.371	** $[M - H]^-$	452.1319	451.1246	451.1247	0.20	289, 273	APE, ASE, * PPE, PSE
					Dihydroflavonols					
29	Dihydroquercetin	$C_{15}H_{12}O_7$	44.268	$[M - H]^-$	304.0583	303.0510	303.0520	3.30	151,125	APE, * ASE
					Flavonols					
30	Procyanidin trimer C1	$C_{45}H_{38}O_{18}$	23.564	** $[M - H]^-$	866.2058	865.1985	865.1992	0.81	739, 713, 695	* APE, APU, ASE, PPE, PPU, PSE
31	Cinnamtannin A2	$C_{60}H_{50}O_{24}$	23.696	** $[M - H]^-$	1154.2692	1153.2620	1153.2600	−1.91	1027, 1001	* APE, ASE, PPE, PPU
32	Procyanidin dimer B1	$C_{30}H_{26}O_{12}$	28.103	** $[M - H]^-$	578.1424	577.1351	577.1324	−4.68	451	* APE, ASE, PPE, PPU, PSE
33	(-)-Epicatechin	$C_{15}H_{14}O_6$	31.218	** $[M - H]^-$	290.0790	289.0717	289.0715	−0.69	245, 205, 179	* APE, ASE, PPU, PSE
34	(-)-Epigallocatechin	$C_{15}H_{14}O_7$	84.626	** $[M + H]^+$	306.0740	307.0813	307.0815	0.65	167, 137	ASE, * PSE
					Flavanones					
35	Eriocitrin	$C_{27}H_{32}O_{15}$	39.899	** $[M - H]^-$	596.1741	595.1668	595.1684	2.69	431, 287	* APE, ASE, PPE, PPU, PSE
36	Naringin 4′-*O*-glucoside	$C_{33}H_{42}O_{19}$	53.036	$[M - H]^-$	742.2320	741.2247	741.2251	0.54	433, 271	APE
					Flavones					
37	Apigenin 7-*O*-glucuronide	$C_{21}H_{18}O_{11}$	20.967	$[M + H]^+$	446.0849	447.0922	447.0910	−2.68	271, 253	* APE, PPE, PPU
38	Isorhoifolin	$C_{27}H_{30}O_{14}$	26.135	$[M - H]^-$	578.1636	577.1563	577.1588	4.33	433, 415, 397, 271	PPU
39	Apigenin 6,8-di-C-glucoside	$C_{27}H_{30}O_{15}$	42.844	** $[M - H]^-$	594.1585	593.1512	593.1535	3.88	575, 503, 473	APE, * ASE, PPE, PSE

Table 2. *Cont.*

No.	Proposed Compounds	Molecular Formula	RT (min)	Ionization (ESI^+/ESI^-)	Molecular Weight	Theoretical (*m*/*z*)	Precursor (*m*/*z*)	Error (ppm)	MS/MS Product Ions	Samples
40	Apigenin 6-C-glucoside	$C_{21}H_{20}O_{10}$	55.256	$[M-H]^+$	432.1056	431.0983	431.0984	0.20	413, 341, 311	PPE
41	6-Hydroxyluteolin 7-*O*-rhamnoside	$C_{21}H_{20}O_{11}$	57.850	$[M-H]^-$	448.1006	447.0933	447.0931	−0.45	285	ASE, * PPE
				Flavonols						
42	Myricetin 3-*O*-arabinoside	$C_{20}H_{18}O_{12}$	10.012	** $[M-H]^-$	450.0798	449.0725	449.0722	−0.67	316	*APU, PSE
43	Myricetin 3-*O*-rutinoside	$C_{27}H_{30}O_{17}$	32.049	$[M-H]^-$	626.1483	625.1410	625.1382	−4.50	301	PSE
44	Myricetin 3-*O*-glucoside	$C_{21}H_{20}O_{13}$	34.017	$[M-H]^-$	480.0904	479.0831	479.0833	0.40	317	APU
45	Quercetin 3-*O*-xylosyl-rutinoside	$C_{32}H_{38}O_{20}$	39.018	** $[M+H]^+$	742.1956	743.2029	743.2060	4.17	479, 317	* ASE, PPE
46	Quercetin 3-*O*-xylosyl-glucuronide	$C_{26}H_{26}O_{17}$	39.195	$[M+H]^+$	610.1170	611.1243	611.1241	−0.33	479, 303, 285, 239	ASE
47	Quercetin 3-*O*-arabinoside	$C_{20}H_{18}O_{11}$	42.798	$[M-H]^-$	434.0849	433.0776	433.0772	−0.90	301	* ASE, PPE
48	Kaempferol 3,7-*O*-diglucoside	$C_{27}H_{30}O_{16}$	43.092	$[M-H]^-$	610.1534	609.1461	609.1478	2.79	449, 287	APE, * ASE
49	Kaempferol 3-*O*-glucosyl-rhamnosyl-galactoside	$C_{33}H_{40}O_{20}$	43.311	$[M-H]^-$	756.2113	755.2040	755.2038	−0.26	285	* APE, ASE
50	Myricetin 3-*O*-rhamnoside	$C_{21}H_{20}O_{12}$	45.428	** $[M-H]^-$	464.0955	463.0882	463.0877	−1.08	316, 271, 221	* ASE, PPE, PSE
51	3-Methoxynobiletin	$C_{22}H_{24}O_9$	61.305	$[M+H]^+$	432.1420	433.1493	433.1505	2.77	403, 385, 373, 345	PPE, * PSE
52	3-Methoxysinensetin	$C_{21}H_{22}O_8$	66.632	$[M+H]^+$	402.1315	403.1388	403.1380	−1.98	388, 373, 355, 327	APE
				Isoflavonoids						
53	6″-*O*-Acetyldaidzin	$C_{23}H_{22}O_{10}$	4.413	$[M-H]^-$	458.1213	457.1140	457.1125	−3.30	221	PPE
54	Sativanone	$C_{17}H_{16}O_5$	9.250	$[M-H]^-$	300.0998	299.0925	299.0914	−3.68	284, 269, 225	APE
55	Dihydrobiochanin A	$C_{16}H_{14}O_5$	11.267	$[M+H]^+$	286.0841	287.0914	287.0905	−3.13	270	PSE
56	3′-*O*-Methylviolanone	$C_{18}H_{18}O_6$	16.973	$[M-H]^-$	330.1103	329.1030	329.1030	0.00	314, 299, 284, 256	PPU
57	Violanone	$C_{17}H_{16}O_6$	20.267	$[M-H]^-$	316.0947	315.0874	315.0868	−1.90	300, 285, 135	PPE
58	3′,4′,7-Trihydroxyisoflavanone	$C_{15}H_{12}O_5$	24.011	** $[M-H]^-$	272.0685	271.0612	271.0623	4.06	177, 151, 119, 107	* APE, PPE, PPU
59	5,6,7,3′,4′-Pentahydroxyisoflavone	$C_{15}H_{10}O_7$	39.465	** $[M+H]^+$	302.0427	303.0500	303.0491	−3.00	285, 257	* APE, PPE, PSE
60	3′-Hydroxygenistein	$C_{15}H_{10}O_6$	42.565	$[M+H]^+$	286.0477	287.0550	287.0560	3.48	269, 259	APE
61	6″-*O*-Acetylglycitin	$C_{24}H_{24}O_{11}$	43.656	$[M+H]^+$	488.1319	489.1392	489.1413	4.29	285, 270	PPE
62	6″-*O*-Malonyldaidzin	$C_{24}H_{22}O_{12}$	45.321	$[M+H]^+$	502.1111	503.1184	503.1189	0.99	255	PSE

Table 2. *Cont.*

No.	Proposed Compounds	Molecular Formula	RT (min)	Ionization (ESI^+/ESI^-)	Molecular Weight	Theoretical (*m*/*z*)	Precursor (*m*/*z*)	Error (ppm)	MS/MS Product Ions	Samples
				Lignans						
63	Episesamin	$C_{20}H_{18}O_6$	20.860	** $[M-H]^-$	354.1103	353.1030	353.1029	−0.28	338, 163	* ASE, PPU
64	Matairesinol	$C_{20}H_{22}O_6$	24.760	$[M-H]^-$	358.1416	357.1343	357.1343	0.00	342, 327, 313, 221	PPU
65	Enterolactone	$C_{18}H_{18}O_4$	35.010	$[M+H]^+$	298.1205	299.1278	299.1292	4.68	281, 165	* PPE, PSE
66	Schisanhenol	$C_{23}H_{30}O_6$	35.468	$[M+H]^+$	402.2042	403.2115	403.2128	3.22	385, 354, 331	SPE
67	Schisandrin	$C_{24}H_{32}O_7$	52.095	$[M+H]^+$	432.2148	433.2221	433.2230	2.08	415, 384, 361	PSE
68	Secoisolariciresinol-sesquilignan	$C_{30}H_{38}O_{10}$	58.039	$[M-H]^-$	558.2465	557.2392	557.2391	−0.18	539, 521, 509, 361	* APE, SPE
				Stilbenes						
69	Piceatannol 3-*O*-glucoside	$C_{20}H_{22}O_9$	8.335	** $[M-H]^-$	406.1264	405.1191	405.1172	−4.69	243	* ASE, PPU
70	4′-Hydroxy-3,4,5-trimethoxystilbene	$C_{17}H_{18}O_4$	29.576	$[M+H]^+$	286.1205	287.1278	287.1270	−2.79	271, 241, 225	*APU, ASE, PPE, PSE
71	Resveratrol	$C_{14}H_{12}O_3$	31.267	$[M-H]^-$	228.0786	227.0713	227.0709	−1.76	185, 157, 143	APE
72	3′-Hydroxy-3,4,5,4′-tetramethoxystilbene	$C_{17}H_{18}O_5$	43.904	$[M+H]^+$	302.1154	303.1227	303.1221	−1.98	229, 201, 187, 175	* PPE, PPU
				Other polyphenols						
				Curcuminoids						
73	Bisdemethoxycurcumin	$C_{19}H_{16}O_4$	77.677	$[M+H]^+$	308.1049	309.1122	309.1137	4.85	291, 263	APE, * PPE
				Furanocoumarins						
74	Isopimpinellin	$C_{13}H_{10}O_5$	17.193	$[M+H]^+$	246.0528	247.0601	247.0595	−2.43	232, 217, 205, 203	APE, APU, PPE, *PPU, PSE
				Hydroxybenzaldehydes						
75	*p*-Anisaldehyde	$C_8H_8O_2$	6.041	** $[M+H]^+$	136.0524	137.0597	137.0601	2.92	122, 109	APE, ASE, * PPE, PPU, PSE
76	4-Hydroxybenzaldehyde	$C_7H_6O_2$	30.767	** $[M-H]^-$	122.0368	121.0295	121.0293	−1.65	92, 77	APE, * ASE, PPE, PPU
				Hydroxybenzoketones						
77	Scopoletin	$C_{10}H_8O_4$	7.554	$[M-H]^-$	192.0423	191.0350	191.0347	−1.60	176, 147	APE, APU, * PPE, PSE
78	2,3-Dihydroxy-1-guaiacylpropanone	$C_{10}H_{12}O_5$	16.950	** $[M-H]^-$	212.0685	211.0612	211.0605	−3.32	167, 123, 105, 93	APE, * ASE, PPE
79	Coumarin	$C_9H_6O_2$	63.127	$[M+H]^+$	146.0368	147.0441	147.0442	0.68	103, 91	PPE, PPU, * PSE

Table 2. *Cont.*

No.	Proposed Compounds	Molecular Formula	RT (min)	Ionization (ESI^+/ESI^-)	Molecular Weight	Theoretical (*m*/*z*)	Precursor (*m*/*z*)	Error (ppm)	MS/MS Product Ions	Samples
				Hydroxyphenylpropenes						
80	2-Methoxy-5-prop-1-enylphenol	$C_{10}H_{12}O_2$	8.404	$[M + H]^+$	164.0837	165.0910	165.0910	0.00	149, 137, 133, 124	* SPE, SPU
				Phenolic terpenes						
81	Rosmanol	$C_{20}H_{26}O_5$	34.541	$[M + H]^+$	346.1780	347.1853	347.1844	−2.59	301, 231	PPU, * SPE
82	Carnosic acid	$C_{20}H_{28}O_4$	80.860	$[M - H]^-$	332.1988	331.1915	331.1922	2.10	287, 269	ASE, * PSE
				Tyrosols						
83	Hydroxytyrosol 4-*O*-glucoside	$C_{14}H_{20}O_8$	14.283	** $[M - H]^-$	316.1158	315.1085	315.1098	4.13	153, 123	ASE, * PSE
84	3,4-DHPEA-AC	$C_{10}H_{12}O_4$	19.522	$[M - H]^-$	196.0736	195.0663	195.0667	2.10	135	APE, * PPE, PSE
85	Demethyloleuropein	$C_{24}H_{30}O_{13}$	38.557	** $[M - H]^-$	526.1686	525.1613	525.1627	2.67	495	* APE, SPE

* Compound was detected in more than one custard apple sample; data presented in this table are from asterisk sample. ** Compounds were detected in both negative $[M - H]^-$ and positive [M+H]+ mode of ionization, while only single-mode data are presented. As shown in the graph, African Pride peel, seed and pulp are abbreviated as APE, ASE and APU respectively; Pink's Mammoth peel, seed and pulp are abbreviated as PPE, PSE and PPU respectively; soursop peel, seed and pulp are abbreviated as SPE, SSE and SPU, respectively.

Dihydroquercetin was the only dihydroflavonol identified in African Pride peel and seed with the typical fragment ion at m/z 151 formed from the cleavage of the flavonoid C ring. The product ion was consistent with that found in a previous study in which dihydroquercetin was characterized in an investigation of the phenolic compounds in custard apple pulp by adopting UPLC-ESI-MS/MS [49].

Compound **33** with $[M - H]^-$ at m/z 289.0715 with fragment ions at m/z 245, m/z 205 and m/z 179 due to the loss of CO_2 (44 Da), flavonoid A ring (84 Da) and flavonoid B ring (110 Da) was characterized as (-)-epicatechin [39]. The research of Huang et al. [50] reported the presence of (-)-epicatechin with identical ionization mode (ESI^-) with precursor ion at m/z 289. Furthermore, the finding in the research of Baskaran et al. [49] was also in accordance with our outcome, as they reported the same product ions for the compound found in the pulp portion. Another identified common compound (Compound **34** showing $[M + H]^+$ at m/z 307.0815) was (-)-epigallocatechin based on MS/MS ions at m/z 167 and m/z 137, corresponding to the RDA fragmentation pattern [51]. Similarly, Baskaran et al. [49] found the presence of epigallocatechin in bound form but in pulp of custard apple rather than the seed as found in the present research. Procyanidin dimer B1 identified at $[M - H]^-$ with m/z 577.1324 was tentatively found in both atemoya peels in our research. The study of Justino et al. [52] reported procyanidin B2 rather than procyanidin B1 in *Annona crassiflora* peel. The findings of Huang et al. [50] explained this variability in their result since two diverse B types procyanidin dimers were identified in the extract of *Annona squamosa* peel, and the m/z values (577 and 579) in negative mode corresponded to the ones reported for procyanidin dimer B2 and procyanidin dimer B1, respectively. Additionally, the results of our study were consistent with those of Justino et al. [52] in the detection of procyanidin trimer C1 in the peel of custard apple samples, while the present study further suggests the presence of this specific compound in the pulp and seed of custard apple.

Flavanone, Flavone and Flavonol Derivatives

For flavanones, all of the constituents shown in Table 2 were reported in custard apple for the first time. Compound **36** detected in negative mode with precursor ion at m/z 741.2251 was identified in African Pride peel and confirmed as naringin 4′-*O*-glucoside with product ions at m/z 433 and m/z 271 [53]. Eriocitrin, a flavanone found in an abundance in citrus fruits [54], was characterized with product ions at m/z 431 and m/z 287 in the negative ionization mode, representing the loss of rhamnose moiety, water and glucose [55].

In terms of flavones, five diverse compounds were characterized in custard apple samples. Compound **37** with $[M - H]^{-m/z}$ at 447.0910 was characterized as apigenin 7-*O*-glucuronide through the comparison with a previous study, with fragment ions at m/z 271 and m/z 253 due to the loss of a glucuronic acid moiety (176 Da) and a water molecule [56]. The presence of product ions at m/z 433 and m/z 271 in negative mode, due to the loss of rhamnose and further cleavage of glucose, identified compound **38** (isorhoifolin), which was uniquely detected in Pink's Mammoth pulp [55]. Compounds **39** and **40** were characterized as apigenin 6,8-di-C-glucoside and apigenin 6-C-glucoside, respectively. Previously, Santos and Salatino [57] reported apigenin 6-C-glucoside as one of the foliar flavones of *Annonaceae* from Brazil, while Kadam et al. [58] illustrated that this compound is widely distributed in medicinal plants and possesses high potential for pharmacological use due to antioxidant and anti-inflammatory properties [59].

Flavonol Derivatives

Myricetin, quercetin and kaempferol derivatives have been characterized in custard apple. Myricetin 3-O-arabinoside (Compound 42), $[M - H]^-$ ion at m/z 449.0722, was tentatively identified in African Pride pulp and Pink's Mammoth seed. The identification was verified by the major produced product ion at m/z 316, indicating the loss of 133 Da (pentose moiety). Previously, this compound was characterized in cranberry cultivars via UPLC-IM–HRMS [60]. Compounds 43, 44 and 50 were also identified as myricetin 3-O derivatives in the present research and were assigned as myricetin 3-O-rutinoside,

myricetin 3-*O*-glucoside and myricetin 3-*O*-rhamnoside, respectively. With respect to quercetin derivatives, all three derivatives could be tentatively identified in African Pride seed. Compound 45 showing precursor ion at m/z 743.2060 was proposed as quercetin 3-*O*-xylosyl-rutinoside, with product ions at m/z 479 and m/z 317 due to the loss of two pentoses and additional loss of a hexose [61]. The identification of quercetin 3-*O*-xylosyl-glucuronide was confirmed by the characteristic fragment ion at m/z 303 produced by the cleavage of glucuronide. The MS^2 spectrum had fragment ions at m/z 285 [M + H–glucuronide–H_2O] and m/z 239 [M + H–glucuronide–2H_2O–CO], further supporting the characterization [62]. Kaempferol 3,7-*O*-diglucoside and kaempferol 3-O-glucosyl-rhamnosyl-galactoside showing $[M - H]^-$ ion at m/z 609.1478 and m/z 755.2038, respectively, were two kaempferol derivatives detected in African Pride peel and seed. The former flavonol gave product ions at m/z 447 and m/z 285 due to the loss of glucose (162 Da) and two glucoses (314 Da), while the latter flavonol yielded characteristic ion at m/z 285, indicating the combination loss of a glucose, a pentose and a glucuronic acid moiety (470 Da) [58,63].

Isoflavonoid Derivatives

A total of 10 isoflavonoid compounds were tentatively identified in custard apples. Sativanone with $[M - H]^-$ ion at m/z 299.0914 was exclusively detected in African Pride peel, exhibiting the fragment ions at m/z 284, m/z 269 and m/z 225 because of the cleavage of CH_3 (15 Da) from the B ring, the loss of two CH_3 (30 Da) and the loss of two CH_3 plus CO_2 (74 Da), respectively [64]. Compound **56** found in Pink's Mammoth pulp with $[M - H]^-$ ion at m/z 329.1030 was suggested to be 3′-*O*-methylviolanone. The MS^2 spectrum of the compound gave product ions in negative mode at m/z 314 [M-H-CH_3▪], m/z 299 [M − H−2CH_3▪], m/z 284 [M − H−3CH_3▪] and m/z 256 [M − H−3CH_3▪−CO] [65]. Compounds **60**, **61** and **62** at positive ionization mode (ESI^+) were assigned as genistein, glycitin and daidzin derivatives and were exclusively detected in APE, PPE and PSE, respectively. In the study of George et al. [1], genistein and glycitein were identified in the aqueous extract of *A. muricata* and daidzein was detected in methanolic extract of *A. muricata* by HPLC.

3.3.3. Lignans and Stilbenes

Lignans and stilbenes are essential classes of polyphenols correlated with plant defense [66]. In total, six lignans and four stilbenes were tentatively characterized in the current research.

Lignan Derivatives

Compound **64** ($[M - H]^-$ at m/z 357.1343) was matairesinol and was detected in Pink's Mammoth pulp. The characterization was confirmed by the product ions at m/z 342, m/z 327, m/z 313 and m/z 221, corresponding to the loss of CH_3, C_2H_6, CO_2 and $C_8H_8O_2$, respectively. Previously, this compound was characterized in *Acanthopanax senticosus* stem [67]. Compound **65** was proposed to be enterolactone identified in Pink's Mammoth seed and peel and had major fragment ions at m/z 281 and m/z 165 because of the loss of water and $C_9H_{10}O$ [68]. Secoisolariciresinol-sesquilignan precursor ion at $[M - H]^-$ at m/z 557.2391 was designated as compound **68**, with product ions at m/z 539, m/z 521, m/z 509 and m/z 361 due to the loss of a single water molecule, two water molecules, $HOCH_2OH$ and guaiacylglycerol, respectively [69]. In addition, two other lignans named schisanhenol (Compound **66**) and schisandrin (Compound **67**) were also tentatively characterized in soursop peel and Pink's Mammoth seed, respectively. The former lignan presented multiple ions at m/z 385, m/z 354 and m/z 331, whereas the latter compound had fragment ions at m/z 415, m/z 384 and m/z 361 due to the loss of H_2O, H_2O-OCH_3 and H_2O-C_4H_6 [70]. Dutra et al. [71] previously isolated three lignans (eudesmin, magnolin and yangambin) from leaves of *Annona pickelii*.

Stilbene Derivatives

To the best of our knowledge, this was the first time that the stilbenes were reported in custard apple. Compound **69** present in negative mode at m/z 405.1172 was confirmed to be piceatannol 3-*O*-glucoside by fragment ion at m/z 243 [M-H-glucoside] [72]. Resveratrol (Compound **71**) was confirmed by the MS^2 spectrum and comparison with the previous literature. The MS^2 spectrum presented fragment ion at m/z 185, corresponding to the loss of CHCOH group, while the other two ions at m/z 157 and m/z 143 were due to the loss of CO and C_2H_2O [73]. According to the literature, stilbenes serve various bioactive functions such as cardioprotection, tumor resistance and bacterial and fungal resistance [74].

3.3.4. Other Polyphenols

The other polyphenols identified in custard apple (a total of 13) can be classified into curcuminoids (1), furanocoumarins (1), hydroxybenzaldehydes (2), hydroxy-benzoketones (3), hydroxyphenylpropenes (1), phenolic terpenes (2) and tyrosols (3).

Compound **79** with $[M + H]^+$ ion at m/z 147.0442 was tentatively characterized as coumarin. According to the MS^2 spectrum, the $[M + H]^+$ ion gave characteristic ions at m/z 103 and m/z 91 due to the elimination of CO_2 and $\cdot HC\text{-}CO_2$, respectively [75]. The presence of coumarin in the bark of *Annona senegalensis* was previously reported in the research by Inkoto et al. [76], while Anaya Esparza and Montalvo-González [59] likewise characterized this compound in the extract of soursop. Sonkar et al. [77] reported the exhibition of liver protective function in rats from the coumarins present in the extract of *Annona squamosa*.

Rosmanol (compound **81** with $[M + H]^+$ at m/z 347.1844) was identified in Pink's Mammoth pulp and soursop peel. The MS/MS spectrum characterized the compound at m/z 301, indicating loss of a water molecule and CO (46 Da) [78]. The presence of phenolic terpenoids in the seed of sugar apple (*Annona squamosa* L.) had already been reported by Huang et al. [50]. Hydroxytyrosol 4-*O*-glucoside (compound **83**) was identified in both positive (ESI^+) and negative (ESI^-) ionization modes yielding the product ions at m/z 153 and m/z 123, corresponding to loss of hexose and a CH_2O group. Previously, Khallouki et al. [79] identified tyrosols in methanolic extract of root and bark of *Annona cuneate*.

Overall, the screening and characterization of phenolic compounds via LC-ESI-QTOF-MS/MS created a profile for the phenolics of custard apple that enables easier estimation and analysis of the antioxidant properties. The outcome displays that custard apple possesses abundant phenolic compounds and indicates that custard apple has a high potential to be used as polyphenol resources in food and pharmaceutical domains.

3.4. Distribution of Phenolic Compounds—Venn Diagram

In the current study, Venn diagrams were plotted to provide additional information on the distribution of phenolic compounds in African Pride, Pink's Mammoth and soursop custard apples. The comparison clearly reveals that the phenolic constituents in different custard apples are diverse, which results in differences in antioxidant potential among the varieties.

Figure 1A shows that a total of 338 compounds were tentatively identified in all nine custard apple samples. Of the total phenolic compounds, 17.5% were possessed by all three custard apples. The percentage for shared phenolic acids in three custard apple fruits was very similar to that of the total phenolics (19%). Unlike phenolic acids, flavonoids have a relatively lower proportion of commonly shared compounds (9.1%), whereas a much higher percentage (27%) was noted for the other phenolic compounds. Based on such low shared proportions, it was apparent that there was a great variation for phenolic constituents in different varieties of custard apples. As shown in Figure 1C, 42.9% of the flavonoid compounds were shared in African Pride and Pink's Mammoth. This result can be explained by both African Pride and Pink's Mammoth belonging to *Annona atemoya*, and the large overlapped zone might be produced due to the shared compounds of this

specific *Annona* species. In the soursop variety, the unique compounds were low when compared to other varieties.

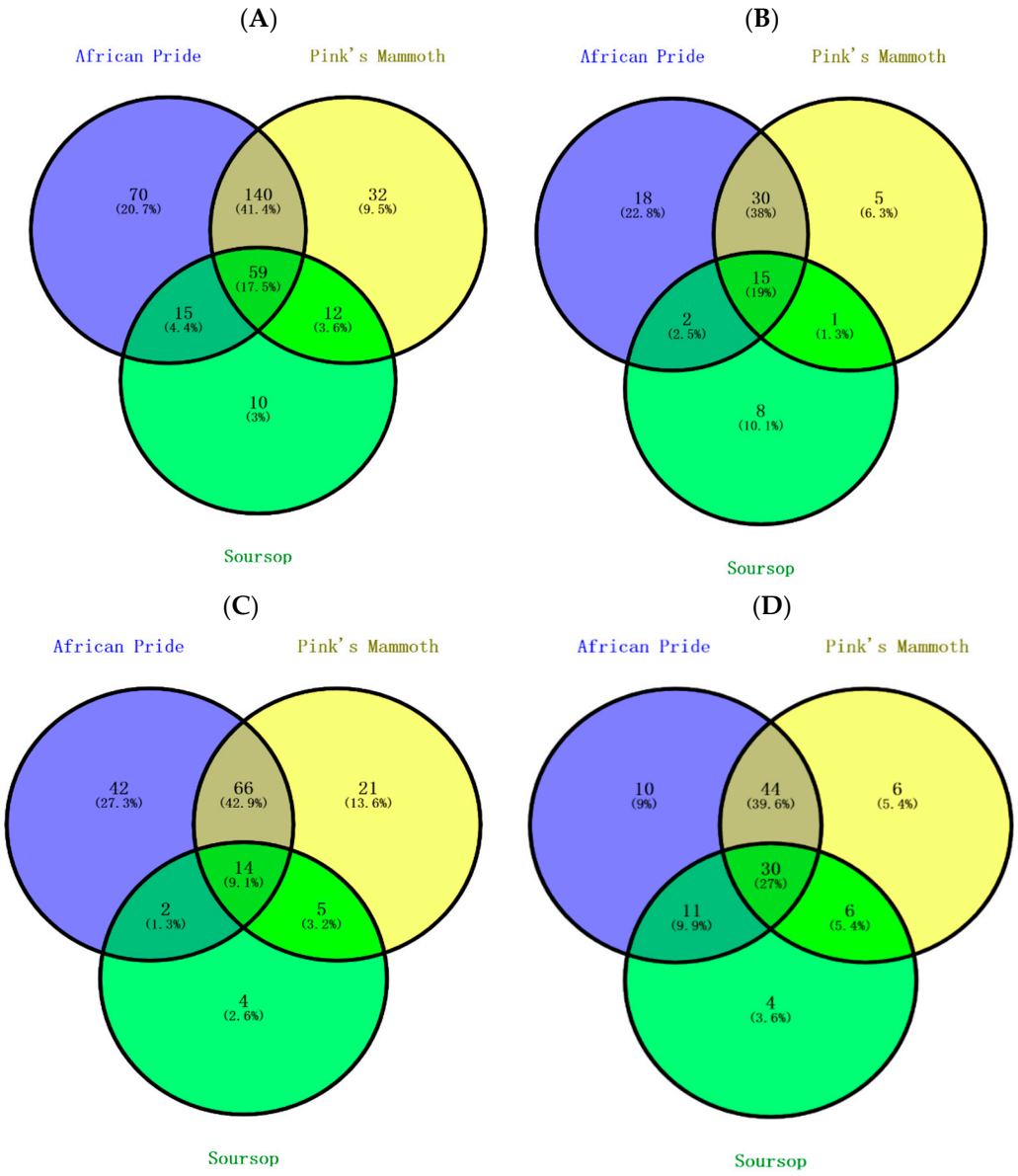

Figure 1. Venn diagram of the distribution of phenolic compounds in various custard apple varieties. The relations of (**A**) total phenolic compounds, (**B**) phenolic acids, (**C**) total flavonoids and (**D**) other phenolic compounds in three custard apples.

As shown Figure 2A, 38.5% of phenolic compounds were shared among the peel, pulp and seed, and 19.5% of compounds were shared between peels and seeds. Considering unique compounds, 17.2% were found in peel, 8.6% in seeds and 3.6% in pulps. The peels have higher levels of phenolic compounds due to the exposure to the outer environment, and this result was previously found in the peel of custard apple [29]. As shown in Figure 2B,C, the highest number of unique compounds was found in African Pride peel and seed, followed by Pink's Mammoth and soursop. Figure 2D shows that the Pink's Mammoth pulp had more unique compounds than African Pride and soursop. The proportions of overlap of the varieties in seed and pulp were 3.8% and 2.7%, respectively. Previously, a study reported that the total phenolic contents were low in soursop, which is similar to our study [6].

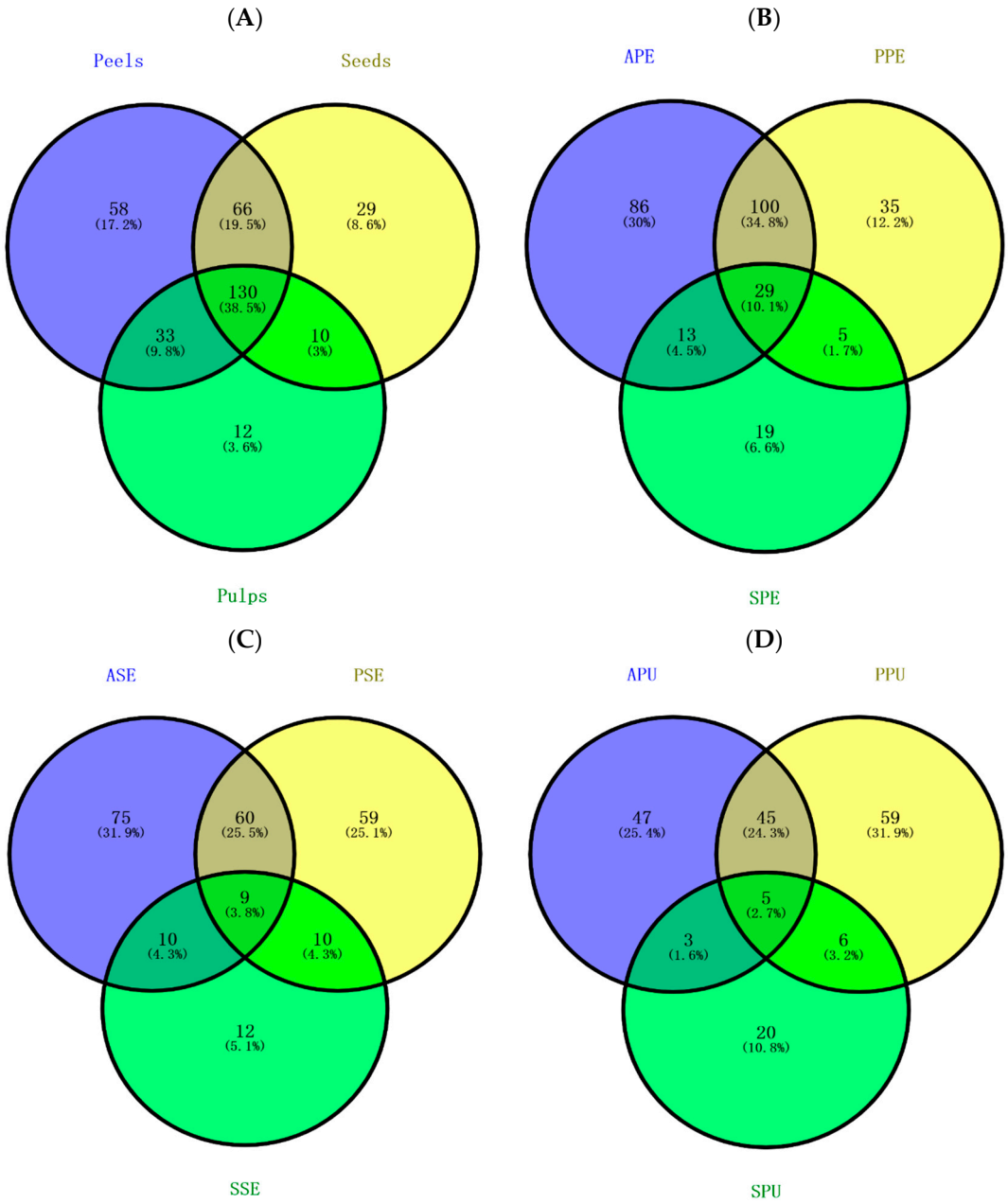

Figure 2. Venn diagram of the distribution of phenolic compounds in various custard apple portions, including peel, seeds and pulp. The relations of (**A**) total phenolic compounds present in three portions of custard apple, (**B**) phenolic compounds in peels, (**C**) phenolic compounds in seeds and (**D**) phenolic compounds in pulps.

The findings in this section further verified the variation of phenolic compounds in different custard apples, and we found that the flavonoids were primarily responsible for the differences. Moreover, the peel sections seem to have numerous compounds among the various portions of custard apples, whereas soursop had the fewest phenolic compounds. Further analysis might be required to investigate the impact of individual phenolic compounds on antioxidant capacity.

3.5. Heat Map and Hierarchical Clustering Analysis of Phenolic Compounds

In the heatmap (Figure 3), there are two clusters in rows and four clusters in columns presented in the form of hierarchical clustering, and each cluster was generated by different samples representing a unique cluster with significant differentials in their phenolic profiles. The color of samples displays whether the contents of the targeted phenolics (phenolic

acids and flavonoids) present in the custard apple samples were relatively abundant. According to the result, PPE, APE, PPU, APU and SSE were grouped into a cluster since they possess a high level of similar phenolic contents. It is worth noting that within this cluster, the contents of quercetin-3-rhamnoside, *p*-hydroxybenzoic acid, syringic acid and quercetin-3-glucoside were high in APE (marked with deep purple color). Apart from the horizontal clustering, phenolic compounds could also be vertically classified into four major clusters (PC-1, PC-2, PC-3 and PC-4) and several sub-clusters that were then distributed based on the concentration similarity in the custard apple samples. The map showed that both quercetin and protocatechuic acid were diverse in terms of the concentration among the samples, while the other phenolic acids and flavonoids tended to have higher similarity.

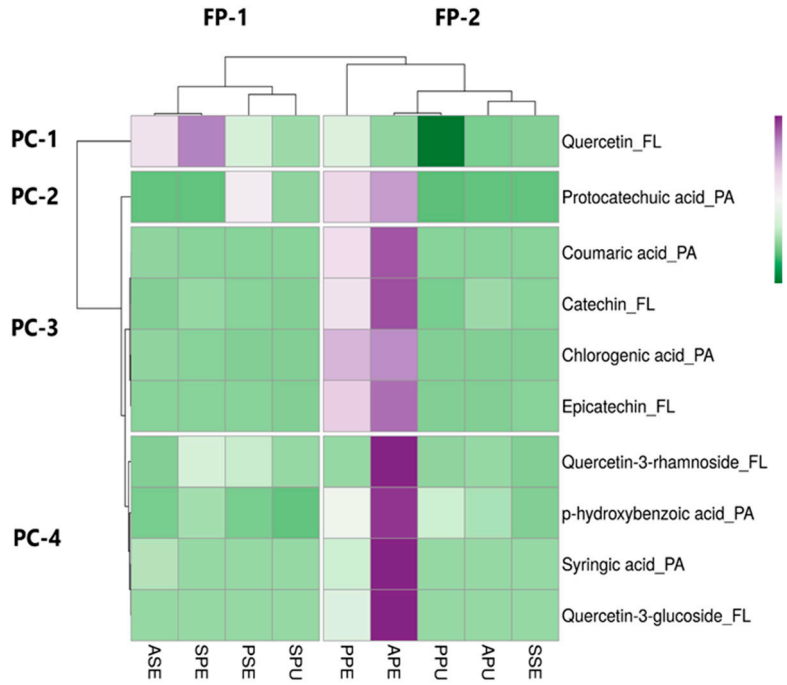

Figure 3. Heatmap displaying the distribution of phenolic compounds and the concentration among custard apple samples. Purple boxes represent the compounds with higher concentrations; green boxes represent the compounds with lower concentrations. African Pride peel, seed and pulp are abbreviated as APE, ASE and APU, respectively; Pink's Mammoth peel, seed and pulp are abbreviated as PPE, ASE and PPU, respectively; soursop peel, seed and pulp are abbreviated as SPE, SSE and SPU, respectively; phenolic acids and flavonoids are abbreviated as PA and FL, respectively; phenolic compound clusters and fruit phenolic clusters are abbreviated as PC and FP, respectively.

Protocatechuic acid was quantified from the peel of African pride variety in our study, and the concentration was relatively high. Protocatechuic acid was also previously reported in the annonaceous fruit called araticum (*Annona crassiflora* Mart.), and the concentration was higher than that found in the African Pride cultivar [80]. The concentration of chlorogenic acid in African Pride peel and Pink's Mammoth peel was comparatively higher than that in African Pride seed and soursop peel. It is worth noting that chlorogenic acid was characterized and quantified in the peel section of all examined fruits, which suggests that perhaps this phenolic compound is abundant in *Annona* fruits. A recent study that measured the chlorogenic acid in the pulp of some edible Annonaceae fruits, including atemoya and soursop, reported a very low concentration of chlorogenic acid, and the compound was not detected in soursop pulp [27]. *p*-Coumaric acid was found in high concentration in the peel of African Pride and Pink's Mammoth. Arruda et al. [80] previously reported a low concentration of *p*-coumaric acid in the peel of araticum fruits. In addition, a negligible amount of *p*-hydroxybenzoic acid was detected in the peel of

Pink's Mammoth, while the compound syringic acid was reported for the first time in custard apple to the best of our knowledge.

With regards to flavonoid, the concentration was low when compared to phenolic acid. Catechin and epicatechin were quantified in Pink's Mammoth seed and African Pride seed. Previously, de Moraes et al. [9] had quantified catechin in atemoya pulp and the reported concentration was 38.6 ± 0.72 $\mu g/g_{d.w.}$, whereas epicatechin was comparatively abundant, showing the concentration of 211 ± 2.2 $\mu g/g_{d.w.}$ in the same sample. Three quercetin derivatives were detected in Pink's Mammoth peel. The concentrations of quercetin-3-galactoside (RT = 39.624 min) and quercetin-3-glucoside (RT = 40.485 min) were similar, while a much lower concentration was reported for quercetin-3-rhamnoside. Alvionita and Oktavia [81] reported the presence of quercetin-3-glucoside in the leaf extracts of *Annona squamosa* L. and indicated that this compound has the properties of an antigout drug due to the strong inhibitory effect on xanthine oxidase. Quercetin was present in both peel and seed portions of Pink's Mammoth. The concentration of quercetin for the araticum peel was similar to our research [80]. The findings in de Moraes et al. [9] showed consistency with our research since no detection was reported in the pulp portion of atemoya and soursop.

3.6. Correlation between Antioxidant Assays and Phenolic Content

Pearson's correlation coefficients showed phenolic content and antioxidant activity were closely correlated, and all antioxidant capabilities were correlative except the DPPH radical scavenging activity (Table 3).

Table 3. Linear correlation coefficients (r^2) for the relationships between phenolics and antioxidant assays.

Variables	TPC	TFC	TTC	DPPH	FRAP	ABTS	RPA	•OH-RSA	FICA	TAC	Phenolic Acids
TFC	0.702 *										
TTC	0.997 **	0.690 *									
DPPH	0.036	0.204	−0.002								
FRAP	0.974 **	0.627	0.986 **	−0.116							
ABTS	0.998 **	0.706 *	1.000 **	0.019	0.983 **						
RPA	0.262	0.709 *	0.249	0.341	0.213	0.269					
•OH-RSA	0.295	−0.233	0.269	0.444	0.209	0.266	−0.492				
FICA	0.034	0.110	0.005	0.119	−0.059	0.015	0.228	0.025			
TAC	0.993 **	0.685 *	0.998 **	−0.027	0.989 **	0.997 **	0.264	0.235	0.017		
Phenolic acids	0.994 **	0.689 *	0.990 **	0.076	0.960 **	0.991 **	0.281	0.316	0.115	0.988 **	
Flavonoids	0.954 **	0.682 *	0.933 **	0.213	0.874 **	0.939 **	0.305	0.401	0.265	0.927 **	0.973 **

** Significant correlation with $p < 0.01$. * Significant correlation with $p < 0.05$.

In brief, TPC had highly significant positive correlations with ferric reducing ability of plasma, total antioxidant capacity and ABTS radical scavenging activity with r^2 values of 0.974 ($p < 0.01$), 0.993 ($p < 0.01$) and 0.998 ($p < 0.01$), respectively. TFC also displayed significant positive correlations with total antioxidant capacity and ABTS radical scavenging activity with r^2 values of 0.685 ($p < 0.05$) and 0.706 ($p < 0.05$), respectively. RPA and TFC showed positive correlation with r^2 value of 0.709 ($p < 0.05$). Similarly, TTC exhibited a highly significant correlation with most of the antioxidant activities assessed in our study, such as ferric reducing ability of plasma ($r^2 = 0.986$) and total antioxidant capacity ($r^2 = 0.998$), but lacked correlation with DPPH radical scavenging activity. Correlations between ABTS radical scavenging activity and total antioxidant capacity were found to be highly interrelated in our research. Manochai et al. [5] reported a strong correlation between TPC and ABTS radical scavenging activity and ferric reducing ability of plasma with r^2 values of 0.958 ($p < 0.01$) and 0.995 ($p < 0.01$), respectively, when analyzing the peel of 10 sugar apples. Nam et al. [43] obtained a negative correlation between DPPH radical scavenging activity, TPC and other antioxidant activities in pawpaw, and their results were similar to our study.

Phenolic acids and flavonoids quantified by HPLC had a significant correlation with the antioxidant activities except for the DPPH, which was similar to the previous correlation in our study. Phenolic acids detected by HPLC had highly significant positive

correlations with ferric reducing ability, total antioxidant capacity and ABTS radical scavenging activity with r^2 values of 0.960, 0.988 and 0.991 at the significance level of 0.01. Similarly, the flavonoids detected by HPLC-PDA were found to be closely correlated with ferric reducing ability (r^2 = 0.874), total antioxidant capacity (r^2 = 0.927) and ABTS radical scavenging activity (r^2 = 0.939). Therefore, it can be concluded that both phenolic acids and flavonoids in custard apple are highly correlated to antioxidant potential.

4. Conclusions

This study is the first to provide the comprehensive phenolic profiles of Australian grown custard apples, and it also examined the phenolic content and antioxidant potential in different sections of custard apples. In conclusion, Australian grown custard apples exhibit promising phenolic contents and antioxidant capacity. According to the results, different portions of the custard apple have high concentrations of phenolic compounds that are closely correlated with strong antioxidant capacity. The application of LC-ESI-Q-TOF-MS/MS technique successfully separated and characterized a total of 85 phenolic compounds in the custard apples, while the HPLC-PDA quantified the most abundant phenolic compounds. The outcome of this study shows that each part of the custard apple could be a good source of phenolic compounds. The peel and seed of custard apple have high phenolic content and strong antioxidant activity; due to their potential value, these portions can be used in the field of food and nutraceutical industries. Future studies, including toxicological and animal studies, may support further application and boost development in relevant industries.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/separations8050062/s1, Figure S1: LC-ESI-QTOF-MS/MS basic peak chromatograph (BPC) for characterization of phenolic compounds of custard apple peel. Figure S2: LC-ESI-QTOF-MS/MS basic peak chromatograph (BPC) for characterization of phenolic compounds of custard apple seeds. Figure S3: LC-ESI-QTOF-MS/MS basic peak chromatograph (BPC) for characterization of phenolic compounds of custard apple pulp.

Author Contributions: Conceptualization, methodology, formal analysis, validation and investigation, J.D., B.Z., V.S. and H.A.R.S.; resources, H.A.R.S., C.J.B. and F.R.D.; writing—original draft preparation, J.D. and B.Z.; writing—review and editing, V.S., B.Z., C.J.B., H.A.R.S. and F.R.D.; supervision, H.A.R.S. and F.R.D.; idea sharing, H.A.R.S., C.J.B. and F.R.D.; funding acquisition, H.A.R.S., F.R.D. and C.J.B. All authors have read and agreed to the published version of the manuscript.

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