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Evaluation of antioxidant activities, total phenolic content (TPC), and total catechin content (TCC) of 10 sugar apple (*Annona squamosa* L.) cultivar peels grown in Thailand

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Abstract

Antioxidant activities, total phenolic content (TPC), and total catechin content were analyzed in the ethanol extracts of 10 sugar apple (*Annona squamosa* L.) cultivar peels grown in Thailand. Antioxidant activity assays carried out in the this study were the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, ferric ion reducing antioxidant power (FRAP), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging activity, and oxygen radical absorbance activity (ORAC). 'Nhur Thong', 'Nung Keaw', and 'Fai Keaw' showed high percentage fruit peel (over 38.0%). 'Nhur Thong', 'Nung Keaw', and 'Fai Keaw' ethanol extracts possessed high antioxidant capacities in the majority of methods used, and thus could be potential candidate of natural antioxidants. 'Fai Keaw' showed the best activity in the ABTS (1.57 mmol of trolox/g dry sample), FRAP (0.43 mmol of trolox/g dry sample), and ORAC assays (2.84 mmol of trolox/g dry sample), as well as the highest content of TPC (140.4 mg) per gram dry sugar apple peel among the 10 cultivars. TPC showed a positive correlation compared with all antioxidant activity assays. In conclusion, 'Fai Keaw' could be utilized as an excellent phenolic compound source for functional food products.

Keywords: *Annona* peel; total catechin content; total phenol content; antioxidant activities.

Practical Application: Thailand sugar apple cultivar showed high antioxidant activity in 10 cultivars tested.

1 Introduction

Because of their potential benefit on the human health, fruits have become increasingly important in human nutrition. The nutritional value and health related characteristics of fruits depend on the concentration of nutrients and phytochemicals as well as on the daily intake and bioavailability (Feliciano et al., 2010; Albuquerque et al., 2016). Fruit waste comprises much of the municipal wastes because urban people consumes fruit in large quantity and food industry discards inedible parts of fruit. As a result, great quantities of fruit residues (principally peels and seeds) are generated in large cities and have become a severe environmental issue (Deng et al., 2015). Thus the effective use of the phytochemicals in fruit residues has been proposed to solve this environmental problem; in addition, this approach could add high value to the fruit residue. Recently, many reports revealed that the contents of natural antioxidants were very high in the peel and seeds of some fruits (Ajila et al., 2007; Okonogi et al., 2007; Vieira et al., 2009; Deng et al., 2012). Tfherefore, it would be beneficial if fruit residues could be utilized to recover natural antioxidants, especially phenolic compounds, thus making them able to be fully exploited in food, pharmaceutical, and cosmetics industries (Makris et al., 2007; Deng et al., 2015).

The sugar apple (*Annona squamosa* L.) is a plant belonging to the family Annonaceae. It is well known for its edible tropical fruit and is mostly distributed in America and Asia (Kumar et al., 2012). The white flesh of *Annona* fruit can be utilized for preparing juices, ice cream, soft drink, and so on (Lima et al., 2010). In Thailand, *A. squamosa* is cultivated in all areas, especially in the northeast part, as a sweet fruit. Many researchers have found that this plant has various beneficial medicinal properties such as antioxidant activities in fruit pulp (Jagtap & Bapat, 2012); antinociceptive, anti-inflammatory activities in leaves (Sousa et al., 2010); and anti-tumor and anti-headlice activities in seeds (Yi et al., 2014). Local Thai *Annona* has been developed by hybridizing with a non-local cultivar to improve production yield. 'Petch Pakchong' is one of the hybrid cultivars that are promoted to Thai farmers because of its outstanding characteristics in terms of high production fruit yield and low tendency to rot after harvesting (Chinnasri & Chinnasri, 2014). Concentrations of bioactive compounds vary considerably depending on the type of plant and cultivar; in addition, several factors affect the concentration of bioactive compounds such as genetic factors, maturity stage, environmental and cultural practices, and post-harvest conditions (Odriozola-Serrano et al., 2008; Albuquerque et al., 2016). The potential of sugar apple peel was reported to be used as a natural antioxidants because of its high phenolic content and antioxidant capacities (Deng et al., 2012; Kumar et al., 2012). Antioxidant capacity reported related

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with total phenolic content, which is expressed as mg of gallic acid/g sample. Mariod et al. (2012) reported that catechin and gallic acid were the main phenolic compounds in the sugar apple peel. However, in case of sugar apple cultivars grown in Thailand, only few information about the relation between antioxidant and major active compounds has been reported. Therefore, in the present study, we aimed to investigate the antioxidant properties in various sugar apple peels from 10 different cultivars grown in Thailand, including 'Fai Keaw', 'Fai Krung', 'Nhung Keaw', 'Nung Krung', 'Nung Thong', 'Pakchong KU-1', 'Pakchong KU-2', 'Petch Pakchong', 'Nhur Thong', and 'African Pride'. In addition, this research can be utilized as a reference for Thai farmers to provide an alternative purpose for planting sugar apples as a functional food source.

2 Materials and methods

2.1 Chemicals

Pure standards of catechin (C), epigallocatechin gallate (EGCG), epicatechin gallate (ECG), epigallocatechin (EGC), epicatechin (EC), caffeine, and gallic acid (GA) were purchased from Sigma Chemical Co. (St. Louis, MO). Folin-Ciocalteu reagent, sodium carbonate, 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid (ABTS$^{\bullet+}$) working solution, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), and fluorescein, 2,2′-azobis(2-amidinopropane) dihydrochloride were also purchased from Sigma Chemical Co. (St. Louis, MO). All other chemicals and solvents used were of analytical regent grade.

2.2 Plant materials

Sugar apple fruits of different cultivars including 'Fai Keaw', 'Fai Krung', 'Nhung Keaw', 'Nung Krung', 'Nung Thong', 'Pakchong KU-1', 'Pakchong KU-2', 'Petch Pakchong', 'Nhur Thong', and 'African Pride' were collected from Pakchong Research Station, Faculty of Agriculture, Kasetsart University, Nakhon Ratchasima, Thailand. The plants were grown in soil from Pakchong (i.e., red color and high iron content). A completely randomized experimental design was applied to the ten cultivars with three replicates. Ripened *A. squamosa* were collected randomly at 100-120 days of age from *Annona* plants that were 5 years old. *Annona* fruits were washed thoroughly with water. Fruit peel, edible parts, and seeds were separated and measured. Fresh peels were then freeze-dried (FDU-1200, Eyela, Japan) for two days, powdered, and stored in plastic bags in desiccators until further analysis.

2.3 Determination of proximate composition

Proximate composition of sugar apple peels was determined using the AOAC methods (Association of Official Analytical Chemists, 2002). Moisture content was analyzed based on weight loss after hot air drying at 105 °C until constant weight, and total ash content was assessed from the weight of the sample after incineration in a muffle furnace at 550 °C. Crude protein content was measured by a micro-Kjeldahl method using 6.25 as the conversion factor. Crude lipids were analyzed by the solvent extraction for 6 h with petroleum ether.

2.4 Extraction of Annona peel

Freeze-dried sugar apple peels from ten cultivars were extracted with 95% ethanol by continuous shaking at 120 rpm and 25 °C for 24 h followed by the filtration through a 0.45 μm membrane, and the resultant filtrate was stored at 4 °C in a refrigerator until further analysis.

2.5 Determination of total phenolic content (TPC)

The TPC in the sugar apple peel extracts (SAPE) was quantified using Folin-Ciocalteu method (by Al-Duais et al., 2009; Ingkasupart et al., 2015). Twenty- microliter aliquots of SAPE were mixed with 100-μL Folin-Ciocalteu reagent. The mixture was incubated for 2 min, followed by the addition of 75 μL of 75 g/L $NaCO_3$ solution and incubated in the dark at 25 °C for 2 h. The reaction mixture was measured by absorbance at 760 nm. The TPC was expressed as mg of GA/g dry *Annona* peel.

2.6 Analysis of catechins and caffeine

SAPE were analyzed for catechins and caffeine based on the method of Khokhar et al. (1997) and Hollman et al. (1999). Briefly, the mobile phases for HPLC separation were 5% acetonitrile (eluent A) and 25% acetonitrile (eluent B) in phosphate buffer (0.025 M, pH 2.4). The gradient employed was as follows: 0-5 min, 15% B; 5-20 min, linear gradient 15-80% B; 20-23 min, 80% B; and 23-25 min, 15% B. Injection volume and flow rate were 10 μL and 1 mL/min, respectively. Stock solutions were prepared using pure standards of C, EC, ECG, EGCG, EGC, and caffeine; 1 mg of each standard was dissolved in 1.0 mL of methanol containing citric acid (80 mg/100 mL). External standards were freshly prepared at the range of 10-100 mg/L for each series of analyses. Detection wavelength was 278 nm. The area and retention times of the analytic peaks were compared with those of respective standards.

2.7 Analysis of gallic acid (GA)

SAPE were analyzed for GA based on previously published methods, using an Agilent 1100 Series HPLC (Agilent Technologies, USA) and a Gemini C18 reversed-phase column (250 × 4.6 mm; Phenomenex Sciences Instrument Co., Ltd., USA). The temperature of the column oven was set at 40 °C. Gradient elution was employed with a mobile phase consisting of methanol (solution A) and 50 mM phosphate buffer, pH 2.5 (solution B) as follows: 70% B, 0-30 min. The flow rate was 1 mL/min (20-μL injection volume) and UV-diode array detection occurred at 280 nm. Spectra were recorded from 200 to 400 nm. External standards of GA (Sigma-Aldrich; 50-200 μg/mL) were freshly prepared for analysis. The area and retention times of the analytic peaks were compared with those of respective standards. GA content was expressed in mg of GA/g dry sample.

2.8 Assays for the estimation of antioxidant activity

ABTS radical cation scavenging activity assay

The ABTS assay was carried out according to the method of Sharma et al. (2008), with slight modifications. An ABTS$^{\bullet+}$ stock solution was diluted with ethanol for working solution until the

absorbance reached 0.70 ± 0.02 at 734 nm. Mixture of 20-µL SAPE and 200-µL $ABTS^{\bullet+}$ working solution was incubated at 25 °C in darkness for 4 min and the absorbance was taken. The SAPE activity was expressed as mmol of trolox/g dry sugar apple peel.

Ferric ion reducing antioxidant power (FRAP) assay

The FRAP assay was carried out based on the methods of Benzie & Strain (1996) and Al-Duais et al. (2009). Twenty-microliter aliquots of SAPE were mixed with 200 µL of FRAP working reagent. The FRAP working reagent was prepared as follows: 300 mM acetate buffer (pH 3.6), 20 mM $FeCl_3.6H_2O$, and 10 mM 2,4,6-Tris (2-pyridyl)-S-triazina in 40 mM HCL were mixed at the ratio of 10:1:1. After incubating the mixture at 25 °C in darkness for 8 min, the absorbance was read at 593 nm. The values were expressed as mmol of trolox/g dry sugar apple peel.

DPPH radical scavenging activity assay

The diluted SAPE of 22 µL was mixed with 200 µL of 150 µM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 95% ethanol followed by the 30 min incubation in the dark at 25 °C and the absorbance was measured at 517 nm (Fukumoto & Mazza, 2000). The percentage scavenging activity of the DPPH radicals was calculated according the following formula: $[(Abs_{blank} - Abs_{sample})/Abs_{blank}] \times 100$, at SAPE concentration of 20 mg/mL.

Oxygen radical absorbance activity (ORAC) assay

Twenty-microliter aliquots of SAPE and 120 µL of fluorescein (70 nM final concentration) were mixed and pre-incubated for 15 min at 37 °C in a Synergy HT Multi-Detection microplate reader (BioTek, Winooski, VT, USA). The reaction was initiated as soon as 60 µL 2,2′-Azobis (2-methylpropionamidine) dihydrochloride (12 mM final concentration) was added For 120 min period the fluorescence values were taken every minute using 485 nm excitation and 528 nm emission filters. The activity of the SAPE was expressed as mmol of trolox/g dry sugar apple peel (Dávalos et al., 2005). The net area under the sample curve was calculated by subtracting the area under the blank curve.

2.9 Statistical analysis

All the assays were carried out in triplicate and mean values reported. Using the SPSS program (version 13; IBM, Armonk, NY, USA), analysis of variance (ANOVA) and Duncan's multiple range test differences between the samples.

3 Results and discussion

3.1 Percent composition of peel, pulp, and seed in sugar apple fruit

The percent compositions of sugar apple fruit peel, pulp, and seed are shown in Table 1. The sugar apple cultivar was divided into four groups based on Thai cultivation (local Thai, Vietnamese, hybrid, and Australian cultivars). The sugar apple cultivars in different groups showed different ratios of fruit peel, pulp, and seed. Nhur Thong, Nung Keaw, and Fai Keaw showed high % fruit peel (>38.0%), whereas Nung Thong showed the lowest % fruit peel (27.0%). Similar to these results, Fai Keaw, Nung Keaw, and Nhur Thong showed relatively high values in antioxidant activities (Table 1). Petch Pakchong showed the highest % pulp (65.9%), which is the reason why this is a commercial cultivar, whereas Fai Krung, Fai Krung, and Nung Keaw showed relatively low % pulp (<57.0%). Nung Keaw displayed the highest % seed (10.2%), while the lowest % seed was found in the African Pride cultivar (2.9%). Petch Pakchong, Nhur Thong, and Pakchong KU1 displayed low % seed (4.1 to 4.5%).

3.2 Proximate composition of freeze-dried peel in sugar apple fruits

The proximate composition of freeze-dried peel in sugar apple fruits is shown in Table 2. The % moisture content ranged from 6.7 to 9.0%. Fai Keaw, Fai Krung, Nung Krung, Petch Pakchong, and African Pride cultivars showed high moisture content (> 8.0%), whereas Nung Thong cultivar had low moisture content (6.7%). For crude protein, African Pride showed the highest % crude protein (8.1%) and Pakchong KU1 had the lowest % crude protein (3.5%) among all the cultivars. The % crude fat ranged between 0.5 and 3.2%, with Nung Krung having the highest crude fat content. In contrast, Fai Krung had the lowest crude fat content. The carbohydrate contents varied between 76.1 and 85.3%. Fai Krung, Nung Keaw, and Pakchong KU1

Table 1. Percent composition of peel, pulp and seed in Annona fruit.

Cultivar	% Fruit peel	% Pulp	% Seed
Fai Keaw	38.9^{a}	52.8^{e}	8.3^{b}
Fai Krung	38.0^{a}	56.1^{d}	6.0^{d}
Nung Keaw	38.2^{a}	51.6^{e}	10.2^{a}
Nung Krung	32.2^{b}	59.9^{c}	7.9^{bc}
Nung Thong	27.0^{d}	64.1^{ab}	8.9^{b}
Pakchong KU1	32.4^{b}	63.5^{b}	4.1^{e}
Pakchong KU2	32.0^{b}	60.9^{c}	7.1^{c}
Petch Pakchong	29.6^{c}	65.9^{a}	4.5^{e}
Nhur Thong	38.7^{a}	56.8^{d}	4.5^{e}
African Pride	32.4^{b}	64.8^{ab}	2.9^{f}

Values with different superscripts in the same column are significantly different at 95% level of confidence based on Duncan's multiple-range test.

Table 2. Proximate composition of freeze-dried peel in 10 cultivar Annona fruits (%, w/w).

Cultivar	Moisture content	Crude protein	Crude fat	Carbohydrate	Total ash
Fai Keaw	8.9^{a}	5.7^{cd}	1.1^{d}	80.9^{b}	3.4^{cd}
Fai Krung	8.8^{a}	5.7^{cd}	0.5^{e}	82.2^{ab}	2.8^{d}
Nung Keaw	7.3^{c}	4.0^{e}	1.0^{d}	85.3^{a}	2.4^{d}
Nung Krung	9.0^{a}	5.3^{d}	3.2^{a}	79.6^{b}	2.9^{d}
Nung Thong	6.7^{d}	4.9^{d}	2.8^{b}	81.0^{b}	4.6^{b}
Pakchong KU1	7.1^{c}	3.5^{f}	1.5^{d}	84.1^{a}	3.8^{c}
Pakchong KU2	7.1^{c}	5.1^{d}	2.4^{c}	79.8^{bc}	5.6^{a}
Petch Pakchong	8.6^{ab}	6.1^{c}	1.4^{d}	79.0^{bc}	4.9^{ab}
Nhur Thong	7.4^{c}	6.5^{b}	1.1^{d}	80.8^{b}	4.2^{b}
African Pride	8.3^{ab}	8.1^{a}	2.3^{c}	76.1^{c}	5.2^{a}

Values with different superscripts in the same column are significantly different at 95% level of confidence based on Duncan's multiple-range test.

displayed the highest % carbohydrate, whereas Pakchong KU2, Petch Pakchong, and African Pride had the lowest. Consistent with the total carbohydrate results, Pakchong KU2 (5.6%), Petch Pakchong (4.9%), and African Pride (5.2%) had high total ash contents, while Fai Krung, Nung Keaw, and Nung Krung showed low total ash contents (under 3.0%).

3.3 TPC, TCC, and gallic acid (GA) content in sugar apple peel extracts (SAPE)

The content of total phenolics, total catechin, and GA in SAPE are presented in Table 3. TPC ranged from 33.8 to 140.4 mg GA/g, TCC ranged from 678 to 1805 μg TCC/g, and GAC ranged from 210 to 1,480 μg GA/g. The highest TPC and TCC values were found in Fai Keaw and Nung Keaw cultivars. Nhur Thong also had a high TCC value. Similar tendency between TPC and TCC was found. Petch Pakchong showed the lowest TPC.

Total phenol content of SAPE was higher than other fruit extracts reported in the literature such as *A. crassiflora* peel (Roesler et al., 2007). Specifically, catechin was reported to be the highest compound among phenolics in the sugar apple pericarp (Wu & Tsay, 1998).

GA from SAPE was separated by reverse-phase HPLC. The elution time of GA was 4 min, with a total run time of 30 min. GA ranged from 210 to 1,980 μg of GA/g dry sample (Table 2). Nhur Thong showed the highest content of GA. Fai Krung and Petch Pakchong showed the lowest content of GA. Even though the Fai Keaw cultivar showed the highest TPC, the contribution of GAC was only 0.28% of total phenolics, as compared to that in Nhur Thong (2.14%). This indicates that the total phenolics of *Annona* peel may be composed of many different compounds.

3.4 Catechins and caffeine content in sugar apple peel extracts (SAPE)

Caffeine and five catechins (EGC, C, EC, EGCG, and ECG) from SAPE was separated by gradient reverse-phase HPLC according to the method of Khokhar et al. (1997).

The results showed that the amount of catechin in SAPE varied between 193.1 and 490.9 μg/g (Table 4). Nung Keaw contained the highest catechin amounts and African Pride the lowest; all showed an overall similar trend among individual catechins (EGC > EC > ECG > EGCG), with the exception of Fai Krung and Nung Krung. Previously study

Table 3. Total phenolic, catechin and gallic acid content of 10 Annona cultivars peel using different assay.

Cultivar name	TPC (mg of GA/g dry sample)	TCC (μg of TCC/g dry sample)	GA (μg of GA/g dry sample)
Fai Keaw	140.4^{a}	1,634^{b}	390^{g}
Fai Krung	43.4^{f}	1,027^{e}	240^{h}
Nung Keaw	108.7^{b}	1,805^{a}	1,630^{b}
Nung Krung	64.1^{e}	954^{g}	440^{g}
Nung Thong	77.9^{d}	1,679^{b}	1,480^{c}
Pakchong KU1	65.6^{e}	985^{f}	760^{e}
Pakchong KU2	78.6^{d}	1,494^{c}	1,060^{d}
Petch Pakchong	33.8^{g}	678^{h}	210^{h}
Nhur Thong	92.6^{c}	1,135^{d}	1,980^{a}
African Pride	67.3^{e}	987^{f}	590^{f}

Values with different superscripts in the same column are significantly different at 95% level of confidence based on Duncan's multiple-range test.

3.5 Antioxidant activities of sugar apple peel extracts (SAPE)

Antioxidant activity of the electron transfer reaction mode

The ABTS and DPPH assays are based on the reduction of $ABTS^{+\bullet}$ and DPPH radicals by the antioxidants present in the plant extracts tested, respectively (Dudonné et al., 2009), whereas, the FRAP assay is based on the reduction of ferric tripyridyltriazine (Fe(III)-TPTZ) to a ferrous complex, which is blue in color (Benzie & Strain, 1996).

The ABTS, FRAP, and DPPH assay results of SAPE from 10 cultivars are shown in Table 5. In the case of ABTS, the antioxidant activity ranged between 0.45 and 1.57 mmol of trolox/g dry sugar apple peel. Among all the cultivars tested, Fai Keaw showed the highest ABTS value, followed by Nung Keaw and Nhur Thong cultivars, whereas the antioxidant activities for Petch Pakchong were the lowest. The FRAP values varied between 0.11 and 0.43 mmol of trolox/g dry sugar apple peel.

Fai Keaw displayed the highest FRAP value of 0.43 mmol of trolox/g dry sugar apple peel. The lowest FRAP value was from the Petch Pakchong cultivar. For the DPPH assay, free radical scavenging activity was expressed as EC_{50} (mg/mL), and ranged between 0.42 and 3.06. Fai Keaw and Nung Keaw showed the highest % inhibition values, followed by Nung Thong and Nhur Thong cultivars. In contrast, the Petch Pakchong cultivar showed the lowest EC_{50} value at 3.06.

Antioxidant activities determined by fluorescent probe and superoxide radicals

Some samples consist of various antioxidant compounds, which display complex reaction kinetics. In this case ORAC assay is found to be particularly useful (Karadag et al., 2009). The results of the ORAC assays conducted on SAPE are listed in Table 5. The antioxidant activities of the ORAC ranged between 1.83 and 2.84 mmol of trolox/g dry sugar apple peel. Fai Keaw and Nung Keaw showed the highest ORAC values, followed by Nung Krung, Pakchong KU2, and Nhur Thong cultivars. In this assay, the Petch Pakchong cultivar showed the lowest antioxidant potential. SAPE from Fai Keaw and Nung Keaw cultivars showed considerably high antioxidant activities in terms of ABTS, FRAP, DPPH, and ORAC values. Several species of *Annona* exhibited excellent antioxidant activity, such as pulp, seeds, and peel of *A. crassiflora* (Roesler et al., 2006, 2007), leaves of *A. dioica* (Formagio et al., 2013), and leaves, bark, roots, and seedcake of *A. squamosa* (Baskar et al., 2007; Mariod et al., 2012).

3.6 Correlation between assays

The relationships between antioxidant assays, TPC, TCC, C, EGC, caffeine, EC, EGCG, ECG, and GAC (Table 6) were analyzed. Our study showed high correlation of TPC, TCC, and EGC with all antioxidant assays compared to C, caffeine, EC, EGCG, ECG, and GAC.

In terms of correlation of EGCG, ECG, and GAC to antioxidant activity values, two different results were observed. In the case of ABTS, ABTS, FRAP, and DPPH assays, relatively high the correlation coefficient was observed. The hydrogen atom transfer assay (ORAC) showed a significantly higher correlation coefficient value than those shown by the electron transfer group (Ingkasupart et al., 2015). This result coincides with the observations of two previous studies. Leopoldini et al. (2004) evaluated the mechanism of the hydrogen atom and electron transfer in phenolic compounds. In the transfer of hydrogen atoms, GA was reported to be one of the most active systems. Rice-Evans et al. (1996) further supported it by showing the effect of the chemical structure of flavonoids and phenolic acids on antioxidant activity.

Table 4. Catechin and caffeine contents of 10 Annona cultivars peel.

Cultivar name	C	EC	EGC	ECG	EGCG	Caffeine
Fai Keaw	398.3^{c}	610.9^{b}	623.2^{c}	1.0^{e}	0.4^{g}	7.4^{f}
Fai Krung	215.1^{f}	182.5^{i}	332.8^{e}	293.0^{b}	3.4^{c}	1.3^{g}
Nung Keaw	490.9^{a}	472.7^{f}	839.8^{a}	1.0^{e}	0.8^{f}	10.3^{ef}
Nung Krung	202.1^{g}	181.5^{i}	239.5^{g}	327.0^{a}	4.1^{c}	1.4^{g}
Nung Thong	444.2^{b}	566.3^{c}	660.7^{b}	7.0^{d}	0.9^{f}	17.4^{d}
Pakchong KU1	386.3^{d}	241.2^{h}	271.0^{f}	80.0^{c}	7.0^{b}	24.6^{c}
Pakchong KU2	382.3^{d}	712.9^{a}	397.8^{d}	ND	0.5^{g}	32.5^{b}
Petch Pakchong	110.0^{h}	533.2^{d}	33.3^{h}	ND	1.3^{e}	61.7^{a}
Nhur Thong	363.8^{e}	334.5^{g}	403.0^{d}	2.0^{e}	32.0^{a}	8.9^{f}
African Pride	193.1^{g}	501.4^{e}	288.9^{f}	1.0^{e}	2.9^{d}	17.7^{d}

C: catechin; EC: epicatechin; EGC: epigallocatechin; ECG: epicatechin gallate; EGCG: epigallocatechin gallate; ND: not detected. All phenolic constituents expressed as μg/g. Values with different superscripts in the same column are significantly different at 95% level of confidence based on Duncan's multiple-range test.

Table 5. Antioxidant activity values of 10 Annona cultivars peel using different assay.

Cultivar name	ABTS	FRAP	DPPH	ORAC
Fai Keaw	1.57^{a}	0.43^{a}	0.42^{a}	2.84^{a}
Fai Krung	0.60^{f}	0.12^{g}	1.78^{e}	2.02^{e}
Nung Keaw	1.30^{b}	0.34^{b}	0.50^{a}	2.80^{a}
Nung Krung	0.74^{e}	0.20^{f}	1.10^{d}	2.60^{b}
Nung Thong	0.95^{d}	0.23^{de}	0.73^{b}	2.46^{c}
Pakchong KU1	0.89^{d}	0.21^{ef}	1.19^{d}	2.22^{d}
Pakchong KU2	1.04^{c}	0.24^{d}	0.85^{bc}	2.57^{b}
Petch Pakchong	0.45^{g}	0.11^{h}	3.06^{f}	1.83^{f}
Nhur Thong	1.28^{b}	0.27^{c}	0.71^{b}	2.56^{b}
African Pride	1.08^{c}	0.22^{de}	0.90^{c}	2.27^{d}

ABTS, FRAP, and ORAC assay expressed as mmol of trolox g^{-1}; DPPH assay expressed as EC_{50} (mg ml^{-1}). Values with different superscripts in the same column are significantly different at 95% level of confidence based on Duncan's multiple-range test.

Table 6. Linear correlation coefficients (*r*) between antioxidant assays, total phenolic content (TPC), total catechin content (TCC), catechin (C), epigallocatechin (EGC), caffeine, epicatechin (EC), epigallocatechin gallate (EGCG), epicatechin gallate (ECG), and gallic acid (GA) contents of 10 sugar apple cultivars, obtained by Pearson's analysis.

	ABTS	FRAP	DPPH	ORAC
TPC	0.958**	0.995**	-0.804**	0.891**
TCC	0.708*	0.755**	-0.746**	0.776**
C	0.704*	0.708*	-0.764**	0.715*
EGC	0.721**	0.753**	-0.761**	0.757**
Caffeine	-0.432	-0.402	0.678**	-0.557*
EC	0.395	0.405	-0.175	0.266
EGCG	0.213	0.031	-0.149	0.071
ECG	-0.515	-0.439	0.204	-0.187
GA	0.498	0.372	-0.567*	0.498

*Significantly correlated at the 0.05 level (2-tailed); **Significantly correlated at the 0.01 level (2-tailed).

4 Conclusions

The present study further supports the view that various SAPE are promising sources of natural antioxidants. Antioxidant properties and TPC differed significantly among the 10 SAPE. Among these SAPE, Fai Keaw and Nung Keaw peel extracts showed very strong antioxidant activities and high TPC. TPC was found to be highly significantly correlated with antioxidant activities, which indicated that phenolic compounds were the major contributor to the antioxidant activities of these SAPEs.

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