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Analysis of antioxidant activities in Mango Peel among different Sri Lankan Cultivars

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Abstract
Mango is a widely consumed seasonal fruit in Sri Lanka which is used in food and beverage industry. Peel of the fruit is a leftover byproduct with a functional food potential. This research study was conducted to evaluate total phenol and total flavonoid contents of 8 mango cultivars. Additionally, two radical scavenging 1,1-diphenyl-2-pireyhydrazyl (DPPH) analysis were used to evaluate the anti-oxidant capacity of mango cultivars. They are Trolox Equivalent Antioxidant Capacity (TEAC) and Ferric Reducing Antioxidant Power (FRAP). Correlations between total phenol and flavonoid were analyzed separately with both FRAP and TEAC. Results have indicated that the mango peels were rich in natural antioxidant compounds and their amount varies slightly among different cultivars. The correlations between total phenol, total flavonoid and FRAP indicated phenolics represent a major part of antioxidant capacity in mango peels. Findings of the study establish a solid foundation for further product development from mango peel.

Keywords: Mango Peel, Total Phenol, Total Flavonoid, FRAP, TEAC

Introduction
Mango (*Mangifera indica* L.) is a tropical fruit widely consumed around the world. Sri Lanka is one of the tropical destinations where there are several locally available mango varieties exist. Mango cultivars grow widely throughout Sri Lanka in dry, intermediate and wet zones. (Kothalawala and Jayasinghe, 2016) [8]. There are lot of advantages of mango as a seasonal fruit due to its nutrient content. It has been found that many fruits and vegetables contain many antioxidant compounds including mango. Phenolic compounds, carotenoids, anthocyanins are known instances for such compounds (Naczk and Shahidi, 2006) [12].
Fruit peels are rich in polyphenolic compounds, flavonoids, ascorbic acid, and this makes them valuable in making antioxidants. There has been increasing focus of attention of many researchers searching for potent antioxidants from mango. According to the studies, different parts of mango, including stem bark, leaves, pulp and peel have different amount of antioxidant compounds varying on many factors such as variety, climatic condition etc. Due to the presence of antioxidants in such parts of the mango plants, it has given promising results for various biomedical activities, including antioxidative qualities, free radical scavenging, anti-inflammatory, and anticancer (Ajila *et al* 2007; Rocha Ribeiro *et al*., 2007; Hernandez *et al* 2007; Percival *et al*., 2006; Ling *et al*, 2009) [1, 14, 5, 9].
Peel is a major by-product of mango processing, and they are discarded as waste. It has been reported that peel was a good source of phytochemicals, such as polyphenols, carotenoids, and it exhibited good antioxidant properties (Ajila *et al* 2007; Kim *et al*, 2010) [1, 6]. It is commonly considered that the concentration and composition of phenolics is affected by genetic, agronomic and environmental factors (Tomás-Barberán and Espín, 2001) [15]. However, the antioxidant activities regarding differences among different mango cultivars have been rarely reported.

Materials and methods
Collection of the samples
Following Sri Lankan mango cultivars were selected to analyze antioxidant activities of the peel at their ripening stage. They were as 'Karthakolomban', 'Willard', 'Malwana', 'Betti Amba', 'Gira Amba', 'Kohu Amba', 'Mee Amba', and 'Dampara' (designed as 1-8 in this research). Only the fruits with minute to no physical damages were selected in an amount of 10 fruits per each cultivar from mango farms in Jaffna Area.
Accumulated mango fruits were cleansed adequately with flowing chlorinated water and their peels were collected for grinding process to separate the extracts. Once grinding process was finished, they were stored in polyethylene vacuum packs at -24°C until they were taken for

required analysis.

Folin-Ciocalteu's (FC) phenol reagent and gallic acid (GA), 1,1- diphenyl-2-pireyhydrazyl (DPPH) and 2,2'-amino-di (2-ethyl-benzothiazoline sulphonic acid-6) ammonium salt (ABTS) radical and all other standards were obtained from reputed chemical manufacturing companies.

Extraction of the sample

As for the initial step, 1g of mango peel of different cultivars was weighed separately and refluxed with 30 ml of 70% methanol at a temperature of 60 °C for 2 h time under magnetic stirring. In the next step, centrifugation was applied to separate the filtrate, and the extraction process was repeated for three times. Collected filtrated was then introduced to concertation under reduced pressure at 40 ºC with a final volume of 30 ml and the obtained solution was then used in the following evaluation and detection.

Determination of total phenolic content and total flavonoid in the extracts

Determination of the total phenol content (TPC) was done by using the FC assay described before with additional modifications (Du *et al*., 2014) [3]. Initially, 0.025 ml of the extract of different cultivar was introduced into test tubes and followed by the addition of 2.0 ml of FC reagent (diluted 10 times with water in advance) and 5.975 ml of water.

Then, the prepared solutions were kept for 5 min at ambient temperature before the addition 2 ml of sodium carbonate solution (7.5% w/v). Next, they were subjected to react in dark for 30 minutes at the same ambient temperature. After preparing the calibration curve from standard gallic acid solutions, the absorbance of the extracted solutions was measured at wavelength of 760 nm on a standard UV–vis spectrophotometer. The results were expressed as milligram gallic acid equivalents (GAE)/g dry weight (fresh weight, FW).

For determination of total flavonoid, Kim's methods were used (Kim *et al*., 2003) [7]. Volume of one milliliter extract solution of different cultivar was mixed with 0.3 ml of 5% $NaNO_2$ and 4 ml of distilled water. Then 0.3 ml of $Al(NO)_3$ was added to the mixture followed by adding 2 ml of 1 M NaOH. Then distilled water was use to dilute the solution up to 10 ml using. The absorbance of the solution was measured at 506 nm and the total flavonoid content was calculated by using a calibration curve of rutin standard and expressed as mg rutin equivalent (QR Equiv)/g FW.

DPPH radical scavenging ability

The method proposed by Liyana-Pathirana *et al.,* (2010) [10] with minor adjustments was used to measure the free radical scavenging activity of the extracts. Method was performed by measuring the decrease in absorbance of DPPH solution at wavelength of 517 nm in the presence of the extracts. The solution of 0.5 mM was prepared by dissolving DPPH in methanol. For the evaluation of free radical scavenging activity, 3 ml of DPPH was added into 0.5 ml of the extracts with different concentrations. The mixture was then allowed to stand at room temperature for 30 min in dark before the absorbance at 517 nm was read. The control was prepared as above without extract. The antioxidant activity was calculated from the following equation and Ao and As of the equation represent the absorbance at 517 nm of the control and sample solution, respectively.

$$\text{Scavenging activity} = \frac{(\text{Ao} - \text{As})}{\text{Ao}} \text{ X } 100$$

Trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP)

Following assay was prepared as per the method reported (Benzie *et al*., 1996) [2]. As the initial step for determining FRAP, the extracts (10 µL) were mixed with 1 ml distilled water and 1.8 ml of the FRAP solution. Then the mixture was allowed to react at a temperature of 37 ºC for 10 minutes. The absorbance of the reaction solution was recorded at a wavelength of 593 nm. Trolox standard solution was used to create the calibration curves and the obtained results were given as µM trolox/g (fresh weight, FW).

TEAC was calculated according to the ABTS scavenging ability of mango peel extract. This was performed by the procedure described by Re *et al* (1999) [13]. For the scavenging of ABTS, 50 µl of different extracts were added to 4 mL of the solution above. Methanol was used as control. After reaction for 10 min, the absorbance was measured at wavelength of 734 nm. Following equation was used to calculate the free radical scavenging ability of extracts.

$$\text{ABTS scavenging activity} = \frac{(\text{Ac} - \text{As})}{\text{Ac}} \text{ X } 100$$

In above equation, Ac and As stands for the absorbance at 734 nm wave length of the control and sample solution, respectively. Trolox standard solution was used to build the calibration curves and the results were expressed in µM trolox/g (FW) units.

Results and discussion

Acquired results of total phenol and total flavonoids in cultivars were indicated in the table 1. Among the 8 cultivars that were evaluated for total phenol content has varied between 2.34 to 15.32, meanwhile the total flavonoid ranged from and 0.77 to 7.08 mg/g, respectively. The cultivar 'Karthakolomban' possess the highest while 'Dampara' possessed the lowest contents of total phenol and total flavonoid. Results in the table indicate that the bioactive compounds in mango peels varied greatly among different Sri Lankan mango cultivars. Similar results have been reported in litchi pericarps (Wang *et al*, 2011) [16].

Table 1: Total phenol, total flavonoid, FRAP and TEAC of different mango peel extracts

Cultivars	1	2	3	4	5	6	7	8
Total phenol (mg/g)	15.32	14.12	10.56	9.74	8.44	6.39	5.58	2.34
Total flavonoid (mg/g)	7.08	6.44	4.19	4.35	4.28	2.25	1.89	0.77
FRAP (µM/g)	135	114	98	85	71	49	40	28
TEAC (µM/g)	185	168	104	78	64	62	53	59

Due to its simplicity and versatility, DPPH is utilized as one of the most common reagents for the evaluation of antioxidant compounds in plant extracts and for other solutions. Radical form of DPPH, a stable state of the molecule, possess the quality of absorbing wavelengths at 517 nm. Due to the above phenomenon, deep violet color of the DPPH will turn in to colorless or pale yellow when neutralizing after the acceptance of an electron or hydrogen radical from an antioxidant in the solution to become a more stable diamagnetic molecule (Matthäus, 2002) [11].

Table 1 has also summarized the values of FRAP and TEAC. It can be observed that FRAP value of mango peel extract of different cultivars ranged from 28 to 135 µM/g, and the turn was the same as that of total phenol and total flavonoid. This was because FRAP represented the total antioxidant activity

of plants, and it was only connected to the total bioactive compounds. The turn of TEAC was not totally in accordance with that of FRAP, and this may be caused due to the same reason of DPPH scavenging ability.

As indicated in figure 1, DPPH scavenging different mango cultivars have not expressed any drastic and significant variations in values as resulted in total phenol and total flavonoid. This may because that the bioactive compounds which could scavenge DPPH might almost be the same in different mango cultivars irrespective of the polyphenols and flavonoids.

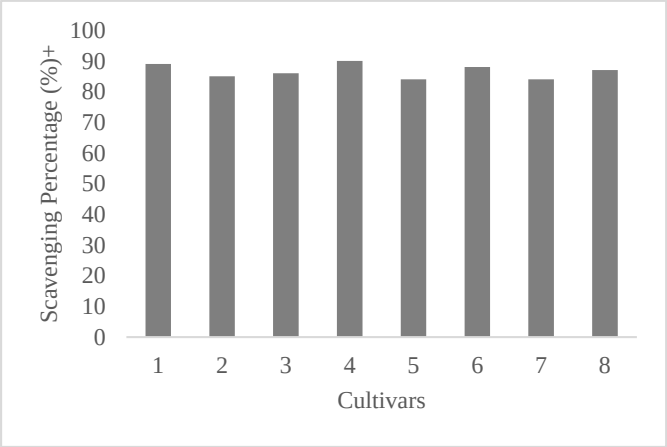
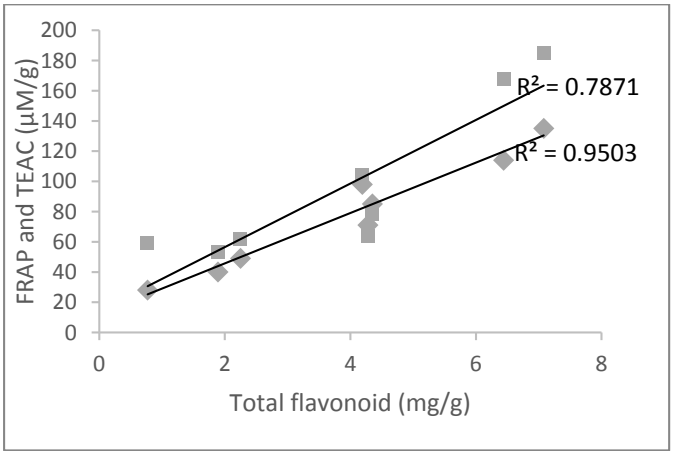


Fig 1: DPPH scavenging abilities of the peel extracts of different mango cultivars.

To determine the contribution of total phenol and total flavonoid to the antioxidant activities of mango peel, FRAP, TEAC values were plotted against them. The correlations obtained were illustrated in Figure 2. Results indicated that correlation between total phenol and FRAP was almost linear (r2=0.97). The trend follows same for the correlation between total flavonoid and FRAP (r2=0.95). These linear relationships conclude that flavonoid and phenol contents in mango peels are directly aligned with total antioxidant quantity. However, the correlation coefficient values between total phenol, total flavonoid and TEAC were relatively lower than the values of FRAP. Correlation coefficient was 0.81 for total Phenol and 0.78 for total flavonoid respectively. This is mainly due to the fact that TEAC were calculated in a different method called ABTS scavenging ability and unlike FARP method, ABTS scavenging ability could not represent the total antioxidant capacity of mango peels. Also, for mango verities with lower values of total phenol and total flavonoid FARP values and TEAC values do not indicate a drastic difference.



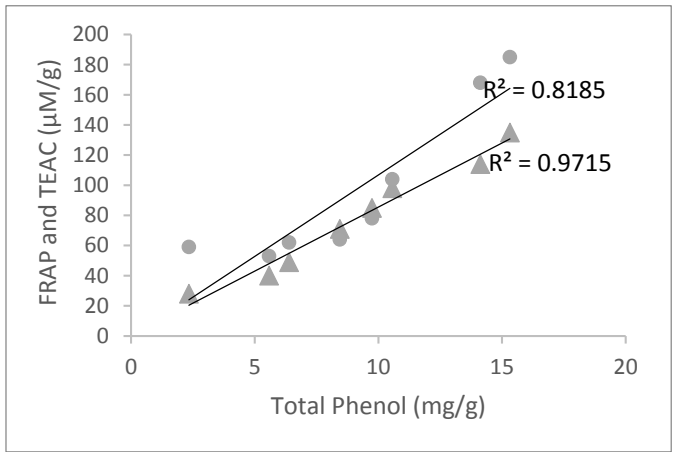


Fig 2: Correlations of (A) total phenol and FRAP, TEAC, (B) Total flavonoid and FRAP, TEAC.

Conclusions

The contents of total phenol and total flavonoid of mango peels of 8 different cultivars, namely 'Karthakolomban', 'Willard', 'Malwana', 'Betti Amba', 'Gira Amba', 'Kohu Amba', 'Mee Amba', and 'Dampara' were obtained and compared in this research. Their antioxidant abilities were also evaluated by DPPH radical scavenging and FRAP. Interpreted results have given the same pattern for both evaluations ('Karthakolomban'> 'Willard'> 'Malwana'> 'Betti Amba'> 'Gira Amba'> 'Kohu Amba'>'Mee Amba'>'Dampara'). Resulted values of FRAP was the same as that of total phenol and total flavonoid, and the highest and lowest were 135 and 28 µM/g, respectively. Results follow the same pattern because FRAP is only connected with all the bioactive compounds to obtain total antioxidant activity of plant extracts. That is the main reason behind the linear correlations of total phenol and total flavonoid for with the FRAP. There were no drastic differences among DPPH radical scavenging abilities of different cultivars, and the turn of TEAC was not completely in accordance with that of FRAP. The cause behind this is mainly because DPPH and TEAC only represented their radical scavenging abilities. The research verified that mango peels were rich in natural antioxidant compounds but their abilities were different among different cultivars due different classes of the antioxidant compounds present in them. Also results have indicated that the phenols acquire a major part of antioxidant capacity in mango peels. This research provides valuable research data for the processed fruit manufacturers in order to make use of the mango peel by product for further product development and reduce waste generation.

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