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Proximate analysis, mineral content and antioxidant capacity of milk apple, malay apple and water apple

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Abstract

The aim of this study is to determine the antioxidant capacity of underutilized fruits in Malaysia namely Milk apple (*Syzygium malaccense*), Malay apple (*Syzygium malaccense* (L.) *Merr*. and Perry), and Water apple (Syzygium aqueum). Synthetic antioxidants (BHA and BHT) commonly used in the food industries may not be as safe as it was presumed earlier. As BHA and BHT may be carcinogenic, it is important to look for new sources of natural antioxidants from fruits and vegetables. Freeze dried samples extracted with acetone and water were measured by ferric 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays. Acetone extract (50%) showed higher values for both DPPH and FRAP assays compared with water extract. Milk apple has the highest DPPH value of 95.26% inhibition of DPPH. Milk apple also showed the highest FRAP value with 8722.22 µM of Fe (II) per gram of freeze dried sample. There was a significant difference ($P < 0.05$) in the types of extraction used. Antioxidant capacities of the samples are in the following order: Milk apple > Malay apple > Water apple. Proximate compositions and mineral contents of the samples were determined too. The samples can be used as a source of natural antioxidants.

Introduction

The fruits of Milk apple (*Syzygium malaccense*), Malay apple (*Syzygium malaccense* (L.) *Merr*. and Perry), and Water apple (*Syzygium aqueum*) were the selected samples of natural antioxidant to be used in this experiment to assess the antioxidant activity of the fruits. All of these fruits were freshly harvested from Kuala Kurau, Perak, Malaysia.

Over the years, consumers have been paying more and more attention to the health and nutritional aspect of horticultural products. Having a diet rich in fruits and vegetables will be able to provide some protection against the common diseases such as cardiovascular diseases, cancers and other age-related degenerative diseases (Scalzo *et al*., 2005). Evidence shows that free radicals are responsible for the damage of lipids, proteins, and nucleic acid in cells could lead to these common diseases (Alothman *et al*., 2009a). Recent studies showed that frequent consumption of fruits and vegetables can reduce the risk of stroke and cancer which is related to the antioxidant microconstituents contained on the plant parts. Different fruits will exhibit different capacities due to the presence of different dietary antioxidants, such as vitamin C and E, carotenoids, flavanoids, and other phenolic compounds (Saura-Calixto and Goni, 2006).

Malaysian population generally consume a lot of tropical and sub tropical fruits in their daily diet that are reported to be high in antioxidant components with strong potential scavenging activities. These tropical fruits are well known with their therapeutic properties and they contain high antioxidants and contribute health benefits to those eating them (Alothman *et al*., 2009a). However, there are some underutilized fruits that grow abundantly in the region of Peninsular Malaysia, Sabah and Sarawak which may have potential benefits towards human health (Ikram *et al*., 2009). Most of these fruits are still growing in the wild or in a semi cultivated state. Reasons why these fruits are classified as underutilized are due to the lack of promotion, minimal planting area, and having an economic potential that has not been fully explored (Shakirin *et al*., 2010).

Butylated hydroxyanisole (BHA, 3-tert-butyl-4-hydroxyanisole) with the E number E320 and butylated hydroxytoluene (BHT, 3,5-di-tert-butyl-4-hydroxytoluene) with the E number E321 are the common synthetic antioxidants used in the food

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industries for human consumption (Conacher *et al*., 1986). They are commonly added into food products such as vegetable oils and snacks to extend their shelf life and prevent damages due to oxidative damages (Leclercq *et al*., 2000).

However, further studies on BHA and BHT showed that these compounds, besides their inhibitory influence upon carcinogenesis, may not be as safe as it was presumed earlier. These synthetic antioxidants may not be rendered completely harmless as BHA promotes the action of some carcinogens while BHT may cause lung damage. Both BHA and BHT were revealed potentially to enhance or even initiate neoplastic process (Hocman, 1988).

Since synthetic antioxidants such as BHA and BHT can be carcinogenic, it is important to find new sources of natural antioxidants especially in fruits and vegetables (Shakirin *et al*., 2010). The replacement of synthetic antioxidants by natural sources may play a role in maintaining health and have benefits for emulsions in food system (Moure *et al*., 2001). Therefore, probably it would be possible to discover more sources of natural antioxidants with the work done on these fruits. The main objective of this study is to evaluate the antioxidant capacity of the fruits, namely using 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) scavenging assay and Ferric Reducing Antioxidant Power Assay (FRAP) method of Milk apple, Malay apple, and Water apple.

Materials and Methods

Chemicals

Chemicals such as acetone, methanol, hydrochloric acid, ferric chloride, ferrous sulphate, sodium acetate, and acetic acid were obtained from R and M Chemicals (Essex, UK). On the other hand, 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Fluka (Switzerland) while 2,4,6-tri(2-pyridyl)-l,3,5-triazine (TPTZ) was obtained from Sigma-Aldrich (St. Louis, MO, USA). All the chemicals used were of analytical grade.

Sample preparation

Freshly obtained Milk apple, Malay apple, and Water apple were separated from its stem and its leaves removed. They were then washed with running water. The fruits were separated into two groups where the first group were blended and the second group were freeze-dried using freeze drier (Alpha 1-2LD Plus, Germany). For blending, the samples were diced into small cubes and blended in a normal grinder (Panasonic MX-7995) for 5 min. These blended samples were then kept in plastic container and refrigerated at 16^{o}C. These fresh samples were not kept for more than 1 week. As for freeze-dried samples, the samples were freeze dried initially and then blended in a normal grinder until they were fine. These samples were then sealed in a plastic bag and kept in and dark, air-tight steel can. The can was then stored in a freezer at -20^{o}C.

Sample extraction

Sample was extracted with modification according to the method by Ikram *et al*. (2009). The extract was obtained by mixing 1 g of sample with 100 ml of 50% acetone (v/v) in a conical flask wrapped with aluminium foil. The mixture was then shaken in an orbital shaker (Lab Companion, Model SI600R) for overnight at 150 rpm and 27^{o}C. The mixture was then centrifuged in a centrifugal at 2500 rpm for 40 min to obtain a clear solution. These steps were repeated using distilled water instead of acetone for water extraction. The extracts obtained were used for the DPPH and FRAP assay.

Proximate analysis

Proximate composition of the samples including moisture (Method 925.40), ash (Method 950.49), protein (Method 955.04), fat (Method 920.39) and crude fibre (Method 935.53) was determined according to the Association of Analytical Chemist (AOAC), 1990 methods. Results obtained were expressed in wet basis.

DPPH free radical-scavenging assay

This method was done as described by Tabart *et al*. (2007) with slight modifications. Stock solution was prepared by mixing DPPH into methanol at a concentration of 100 µmole/L. The DPPH solution at the amount of 6 ml was added to 1 ml of properly diluted sample extract. Control was prepared by mixing 6 ml of DPPH with 1 ml of methanol. The mixture was then vortexed for 1 min and kept in the dark for 30 min. Absorbance was taken at 517 nm of wavelength using UV-Vis spectrophotometer (Shimadzu UV-Visible Recording Spectrophotometer Model UV-160A) against blank. The results obtained were calculated and expressed in the terms of % DPPH inhibition.

Ferric reducing antioxidant power assay (FRAP assay)

This assay has been used because it is a simple and inexpensive method to measure the total antioxidant levels in the samples (Griffin and Bhagooli, 2004). Based on the method proposed by Alothman et al. (2009b), a modified method of FRAP assay was performed. An amount of 200 µL aliquot of properly diluted sample extract was mixed with 3 ml of

FRAP reagent. A blank sample was prepared using distilled water and both of the sample and the blank were incubated in a water bath for 30 min at 37°C. The absorbance of sample was determined against blank at 593 nm of wavelength. The FRAP reagent was prepared fresh by mixing 10 mM 2,4,6-tris (1-pyridyl)-5-triazine (TPTZ) solution in 40 mM HCl with 20 mM $FeCl_3$. $6H_2O$ and 0.3 M acetate buffer, pH 3.6 in a ratio of 1:1:10 and pre warmed at 37°C. A standard curve was prepared using ferrous sulphate $FeSO_4$. $7H_2O$. The values obtained were expressed on a freeze dried basis in µM of ferrous equivalent Fe (II) per gram of freeze dried sample.

Mineral determination

Minerals content of samples such as calcium (Ca), zinc (Zn), iron (Fe), sodium (Na), manganese (Mn), copper (Cu), nickel (Ni), chromium (Cr), lead (Pb) and cadmium (Cd) were determined using atomic absorption spectroscopy (AAS). Absorbencies obtained were recorded and standard curve was plotted. Results obtained were in the unit of mg/g of freeze dried sample.

Statistical analysis

All the results obtained were as means ± SD. One-way analysis of variance (ANOVA) was used to determine the significant differences for multiple comparisons which was completed using Duncan test at $\alpha = 0.05$. All of these were done using SPSS statistical package (ver.17.0).

Results and Discussion

Proximate analysis

Proximate composition which includes moisture, ash, fat, protein, crude fibre and carbohydrate of Milk apple, Malay apple, and Water apple fruits are shown in Table 1. Fresh samples were used in this part of the study. The determination of the proximate compositions was done in duplicates. All the data obtained were from wet basis and expressed in percentage (%).

Moisture content of the samples was significantly different among themselves at $p < 0.05$. Moisture content of Milk apple, Malay apple, and Water apple was generally very high with all of them having more than 80%. The water content of Milk apple was 88.38%, Malay apple 83.28% and Water apple 89.82%. The high content of moisture in the samples suggested that they have high perishability (Adeleke and Abiodun, 2010).

There was a significant difference in the ash content between all the samples at $p < 0.05$ as well.

Table 1. Proximate composition of the samples

Samples	Milk apple (%)	Malay apple (%)	Water apple (%)
Moisture	88.38 ± 0.15[b]	83.28 ± 0.16[a]	89.82 ± 0.32[c]
Ash	0.49 ± 0.05[b]	0.85 ± 0.06[c]	0.33 ± 0.01[a]
Fat	0.24 ± 0.01[a]	0.30 ± 0.03[ab]	0.37 ± 0.03[b]
Protein	0.43 ± 0.03[b]	1.21 ± 0.09[c]	0.12 ± 0.03[a]
Crude Fibre	1.81 ± 0.09[b]	1.68 ± 0.03[b]	0.86 ± 0.01[a]
Carbohydrate	8.65 ± 0.05[a]	12.68 ± 0.25[b]	8.49 ± 0.37[a]

[a] Values are means (n = 2) ± SD.
[b] Values with different superscript are significantly different at $p < 0.05$

The amount of ash in the samples was generally low which includes metal salts and trace minerals. The amount of ash in Milk apple was 0.49% followed by 0.85% in Malay apple and 0.33% in Water apple as well. The amount of ash present can be translated to the quantity of minerals present in the samples (Coimbra and Jorge, 2011).

Fat content in the samples was very low overall which is common for fruits. The fat content ranges from 0.24% to 0.37% with a significant difference only between Milk apple and Water apple at $p < 0.05$. They were lower compared to the fat content in Dragon Fruit (Hylecereus polyhizus) reported by Ruzainah et al. (2009), which was 4.5% for freeze-dried sample and 5.5% for oven dried sample. However, there was no significant difference at $p > 0.05$ between Malay apple and the other two samples.

There was a significant difference at $p < 0.05$ in the protein content between the three samples with the protein content ranging in between 0.12% to 1.21%. Malay apple has the highest content of protein with 1.21%, which was higher than the protein content in Thai seedless guava juice as reported by Shamsudin *et al*. (2005) with 0.80%.

As for fibre content, the samples generally contain 0.86% to 1.81% of crude fibre. Nevertheless, these values only indicate a part of the actual dietary fibre available in the samples (Heller and Hackler, 1978). Crude fibre was present in the largest amount in Milk apple with 1.81%. In addition, there was a significant difference between Water apple and the other 2 samples at $p < 0.05$.

Last but not least, significant difference was found between Malay apple and the other two samples in their carbohydrate content at $p < 0.05$. The carbohydrate content ranged from 8.49% to 12.68% which is low and cannot be a good source of energy (Adeleke and Abiodun, 2010). Malay apple has the highest amount of carbohydrate with 12.68%.

Minerals determination by AAS

Minerals such as calcium, zinc, ferum, sodium,

Table 2. Mineral content of the samples

Minerals \ Samples	mg per g of freeze dried sample		
	Milk apple	Malay apple	Water apple
Calcium	0.399 ± 0.002[b]	0.310 ± 0.0001[a]	0.636 ± 0.004[c]
Zinc	0.019 ± 0.003[a]	0.029 ± 0.000[b]	0.020 ± 0.003[a]
Ferum	0.030 ± 0.001[a]	0.092 ± 0.002[c]	0.037 ± 0.003[b]
Sodium	1.968 ± 0.044[c]	0.895 ± 0.043[b]	0.196 ± 0.008[a]
Manganese	0.032 ± 0.0004[a]	0.037 ± 0.0009[b]	0.033 ± 0.0003[a]
Nickel	0.044 ± 0.004[ab]	0.050 ± 0.006[b]	0.039 ± 0.003[a]
Chromium	0.016 ± 0.001[a]	0.012 ± 0.003[a]	0.014 ± 0.004[a]
Cadmium	0.047 ± 0.001[a]	0.044 ± 0.006[a]	0.046 ± 0.006[a]

[a] Values are means (n = 3) ± SD.
[b] Values with different superscript are significantly different at $p < 0.05$

Table 3. DPPH inhibitions (%) of the samples using different solvent extraction

Samples	% inhibition of DPPH	
	Acetone Extraction	Water Extraction
Milk apple	95.26 ± 0.07[e]	60.62 ± 6.99[c]
Malay apple	86.80 ± 0.80[d]	24.26 ± 2.18[a]
Water apple	94.91 ± 0.07[e]	37.20 ± 3.69[b]

[a] Values are means (n = 3) ± SD.
[b] Values with different superscript are significantly different at $p < 0.05$

manganese, nickel, chromium, cadmium, lead, and copper were detected using atomic absorption spectroscopy (AAS). Standard curves were plotted using absorbencies that were obtained. The results obtained are shown in Table 2 as the mean and standard deviation. Freeze dried samples were used throughout this part of the study and the results obtained were expressed in terms of milligrams (mg) per gram (g) of freeze-dried sample. The determination of the mineral content was done in triplicates. In all the 3 samples, for every 1 kg of fresh sample being freeze dried, approximately 100 g of freeze-dried sample was obtained. This produces a yield of about 10%. Minerals such as calcium, ferum, and sodium are essential in maintaining a good health. Besides that, zinc plays quite a crucial role as well. There has also been an increasing concern in the amount of minerals in food as human's fundamental minerals (Arslan and Özcan, 2008).

The table shows that sodium was the largest amount in Milk apple with 1.968 mg/g and as well as Malay apple with 0.895 mg/g. However, calcium was the mineral that was in the largest amount for Water apple with 0.636 mg/g. There was a significant difference at $p < 0.05$ between all of the 3 samples in the content of calcium with values ranging from 0.310 mg/g to 0.636 mg/g, ferum from 0.030 mg/g to 0.092 mg/g and sodium from 0.196 mg/g to 1.968 mg/g. As for zinc and manganese, there was a significant difference between Malay apple and the other 2 samples at $p < 0.05$. Significant difference was only found between Malay apple and Water apple at $p < 0.05$ for nickel content with values ranging from 0.039 mg/g to 0.050 mg/g.

However, there was no significant difference at $p > 0.05$ between all the 3 samples in their content of chromium with values ranging from 0.012 mg/g to 0.016 mg/g and cadmium from 0.044 mg/g to 0.047 mg/g. Chromium also was the mineral with the lowest quantity found in all 3 samples. Lastly, lead and copper were not detected in all the samples. The samples in general have different composition of mineral contents as they depend on their species. Other factors such as type of soil, climate and season as well as the water used would play a role (Steven *et al*., 1985).

DPPH assay

The ability of inhibition of DPPH in 3 different fruits samples extracted using acetone and water was studied. Table 3 shows the results obtained in terms of their mean and standard deviation. Freeze-dried samples were used throughout this part of the study and the results obtained were expressed in terms of percentage (%) inhibition of DPPH with absorbance read at 517 nm. The mixture was then vortexed for 1 min and kept in the dark for 30 min. Negative control was prepared mixing methanol and DPPH. The determination of the percentage (%) inhibition of DPPH was done in triplicates. Freeze-dried samples were used as they have better extraction effectiveness. This may be caused by the rupture of cell structure due to ice crystals formed inside the plant matrix which enables the cellular components as well as surplus of solvent to leach out (Chan *et al*., 2009).

According to Guo *et al*. (2003), fruits generally have a wide range of antioxidant composition as well as antioxidant capacity. Fruits with higher antioxidant capacity are assumed to have higher amount of antioxidants. The DPPH radicals are organic nitrogen radical that is stable and they could be obtained commercially from the market. The percentage (%) inhibition of DPPH obtained within the assay time in general reflects the antioxidant capacity of the samples. The assay time would usually range from 10-20 min but could be up to 6 hr (Alothman *et al*., 2009b).

Overall, the result shows that all of the samples exhibit antioxidant activity. It can be seen from the table that overall Milk apple has the highest percentage (%) inhibition of DPPH using acetone extraction with 95.26% or water extraction with 60.62%. There was a significant difference between Malay apple and the other 2 samples using acetone extraction at $p < 0.05$. On other hand, when extracted using water, all the samples were significantly different at $p < 0.05$. Malay apple has the lowest percentage (%) inhibition of DPPH in acetone and water extraction. In acetone extraction, the percentage (%) inhibition of DPPH was 86.80% while in water extraction it was 24.26%. The percentage (%) inhibition of DPPH of Milk apple and Water apple were slightly higher than those of

Table 4. FRAP values of the samples using different solvent extraction

Samples	µM Fe(II) per g of freeze dried sample	
	Acetone Extraction	Water Extraction
Milk apple	8722.22 ± 814.68[d]	2436.11 ± 170.24[b]
Malay apple	2062.78 ± 115.29[b]	378.33 ± 75.35[a]
Water apple	3545.56 ± 194.29[c]	847.22 ± 133.60[a]

[a] Values are means (n = 3) ± SD.
[b] Values with different superscript are significantly different at $p < 0.05$

pineapple and banana (pisang mas) and higher than guava in the study by Alothman *et al*. (2009b) using the same solvent.

In general, samples extracted with acetone have a higher percentage (%) inhibition of DPPH compared to samples extracted with water. There is a significant difference at $p < 0.05$ between the types of extraction solvent used and the percentage (%) inhibition of DPPH which suggest the different type of solvent used for extraction would result in different antioxidant capacity. Based on Sultana *et al*. (2009), the type of solvent used for extraction will result in the amount of antioxidant obtained due to chemical characteristics and polarities. Among the common choices used for extractions are aqueous mixtures of ethanol, methanol, acetone, or ethyl acetate. A study by Tabart *et al*. (2009) also suggested that samples extracted with acetone give a higher antioxidant capacity when measured using DPPH.

Alothman *et al*. (2009b) showed that the type of solvent used for extraction and its concentration will affect the antioxidant capacity. In their study, percentage (%) inhibition of DPPH of pineapple, banana (pisang mas) and guava extracted with methanol, ethanol, and acetone are generally higher than those extracted with water. The percentage (%) inhibition of DPPH increases with increasing concentration of the solvent used. However, the percentage (%) inhibition of DPPH of guava which is from the same family as the samples extracted with water was higher compared with guava extracted with methanol at any concentration in the study by Alothman *et al*. (2009b) suggested the use of methanol would not be the best choice of solvent to extract antioxidants. The results obtained by Alothman *et al*. (2009b) also suggested that acetone would be a better solvent to extract antioxidants from fruits.

There are some disadvantages related to the use of DPPH assay. Complications might arise to interpret test compounds, for example, carotenoids with spectra that overlap those of DPPH at 515 nm. With DPPH being both the radical probe and oxidant, this has caused the assay to be not a competitive reaction. Radical reaction, reduction and even non related reactions could have decolourized DPPH with the reaction's primary determinant being steric accessibility. Therefore, DPPH could be decolourized by both reducing agents and hydrogen ions. Besides that, many antioxidants may be unable or react slowly with DPPH due to steric hindrance although they could react rapidly with peroxyl radicals. All of these would result in inaccurate understanding of the total antioxidant capacity (Prior *et al*., 2005).

FRAP assay

In this part of the study, the amount of Fe (II) produced was measured in 3 different fruits samples extracted using acetone and water. Ferrous sulphate ($FeSO_4.7H_2O$) was used to produce a standard curve and the absorbencies obtained were plotted producing equation $y = 0.0006x + 0.1225$ with $r^2 = 0.9944$. The results obtained were shown in Table 4 expressed in mean and its standard deviation. Freeze-dried samples were used throughout this part of the study and the results obtained were expressed in the terms of µM of ferrous equivalent Fe (II) per g of freeze-dried sample with absorbance read at 593 nm. FRAP reagent used was freshly prepared and pre-warmed at 37 °C. The amount of µM of Fe (II) per g of freeze-dried sample was measured in triplicates.

Methods used to determine antioxidant capacity usually involve the ability of the antioxidants in scavenging of certain radicals, inhibiting lipid peroxidation and chelating metal ions. Plant materials are usually studied using FRAP assay to determine its antioxidant capacity (Alothman *et al*., 2009b). Antioxidants or reductants can only be evaluated directly using FRAP assay. Antioxidants will react with ferric tripyridyltriazine (Fe^{3+}–TPTZ) complex to produce a blue ferrous tripyridyltriazine (Fe^{2+}–TPTZ) that will determine the antioxidant capacity of samples. Results obtained in FRAP assay generally represent all the electron-donating reductants in the samples (Abu Bakar *et al*., 2009).

There was a report by Kaur and Kapoor (2001) related to the limitations in methodology to determine antioxidant capacity. Common methods used to determine antioxidant capacity includes radical species production. The amount of radicals disappeared will determine the amount of antioxidant present. It would be crucial to determine and compare the antioxidant capacity with another method rather than depending just on one method (Ikram *et al*., 2009). Therefore, antioxidant capacity of the samples was determined using FRAP assay after they were determined with DPPH assay.

This study has found that all of the samples showed antioxidant activity. Whole fruits were freeze-dried with everything intact as a study done by Shakirin *et al*. (2010) which showed that samples with skin and flesh will have higher antioxidant capacity as there might be possibility of the presence

of potent antioxidant compounds. It can be seen from Table 4 that Milk apple contained the highest amount of μM of Fe (II) per g of freeze-dried sample with 8722.22 μM using acetone extraction and 2436.11 μM using water extraction. On the other hand, Malay apple has the lowest amount of μM of Fe (II) per g of freeze-dried sample with 2062.78 μM using acetone extraction and 378.33 μM using water extraction. All of the samples were significantly different among themselves at $p < 0.05$ when extracted using acetone. However, when extracted with water there was only significant difference at $p < 0.05$ between Milk apple and the other 2 samples.

The FRAP value of Malay apple obtained was much higher compared with the result obtained from Ikram *et al*. (2009) with a value of 220 μM with sample being extracted with 80% methanol. There was a significant difference between the type of solvent used and the amount of μM of Fe (II) per g of freeze-dried sample. It also could be seen in general samples extracted with acetone have a higher amount of μM of Fe (II) compared with samples extracted with water. The FRAP value of the all of the samples extracted with acetone were generally higher than the tropical fruits in the study by Rufino *et al*. (2010). Camu-camu was the only sample in the study with FRAP value slightly higher than Malay apple. The FRAP values of Milk apple, Malay apple and Water apple extracted with acetone too were very high compared with fruit samples reported by Fu *et al*., (2011).

Based on a study by Alothman *et al*. (2009b), pineapple, banana (pisang mas) and guava extracted using either ethanol, methanol or acetone at any concentration have FRAP values that were higher than samples extracted with water. The FRAP values generally increase with the increasing concentration of the solvent. It is worth noting that guava, which is from the family Myrtaceae, and similar to Milk apple, Malay apple, and Water apple exhibit very high antioxidant activity compared with the other samples. However, based on the study by Alothamn *et al*. (2009b) guava extracted with any concentration of methanol gives FRAP values that are lower than guava extracted with water which was similar to the results obtained in DPPH assay.

Nevertheless, FRAP assay has its own limitations. There can be a great variation in the FRAP values obtained as a result of the time scale of analysis. For example, short reaction time of about 4 min would be best for phenols that are fast-reacting while longer reaction time of about 30 min was needed to obtain optimum results in slow-reacting polyphenols. Besides that, thiol antioxidants, for example, gluthathione cannot be measured using FRAP assay. As the assay only measures the ability to reduce ferric ion, it is considered to be not practical to antioxidant mechanistically and physiologically (Prior *et al*., 2005). Lastly, based on a study by Pulido *et al*. (2000), the absorption of polyphenols increased slowly at 593 nm even after several hours of reaction time. Therefore, a completed reaction cannot be obtained from a single-point absorption endpoint.

Conclusion

The main composition of the samples was water with all of the samples having moisture content above 80%. Other components such as ash, protein, fat and fibre present at low amount while carbohydrate present at an appreciable amount within all the samples. All the fruits generally contain low amount of minerals with most of them not having a significant difference in mineral content between the samples at $p < 0.05$. Milk apple consistently showed the highest percentage (%) inhibition of DPPH as well as FRAP value using both acetone and water extraction. This shows that there is a good correlation between DPPH and FRAP assay. All the samples can be a very good source of natural antioxidants. Furthermore, these local fruits have the potential to be used in processed food as natural antioxidants.

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