Journal of Engineering and Applied Sciences 14 (4): 1169-1177, 2019
ISSN: 1816-949X

Underutilized Mangifera Species (*Mangifera caesia, Mangifera quadrifida and Mangifera odorata*) from Borneo: A Potential Source of Natural Antioxidant

[1, 2]Nur Amalina Ismail, [1, 2]Mohd Fadzelly Abu Bakar, [1, 2]Fazleen Izzany Abu Bakar,
[1]Azlen Che Rahim and [3]Norizati Murdin
[1]Faculty of Applied Sciences and Technology (FAST),
Universiti Tun Hussein Onn Malaysia (UTHM), Hub Pendidikan Tinggi Pagoh,
KM 1, Jalan Panchor, Muar, 84600 Johor, Malaysia
[2]Centre of Research for Sustainable Uses of Natural Resources (CoR-SUNR),
Universiti Tun Hussein Onn Malaysia (UTHM), Parit Raja, Batu Pahat, 86400 Johor, Malaysia
[3]Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah (UMS), Jalan,
Kota Kinabalu, 88400 Sabah, Malaysia

Abstract: The search for natural antioxidant in replacement to synthetic antioxidant is still on going. Natural antioxidant displayed a wide range of biological and therapeutic activity which could be obtained from fruits, vegetables, nuts, flowers and bark of tree. Fruits produced by *Mangifera* species are among the underutilized tropical fruits. Most of the research for this genus focused on popular species such as common mango (*Mangifera indica*). This study was conducted to determine the phytochemical content and antioxidant activities of different parts of the fruits (peel, flesh and seed) of selected underutilized Borneo wild *Mangifera* species, i.e., Bundu (*Mangifera caesia*), Dumpiring (*Mangifera quadrifida*) and Mangga Wani (*Mangifera odorata*). Spectrophotometric method was used to determine the phytochemicals content (total phenolic, total flavonoid, total anthocyanin and total carotenoid contents) and antioxidant activities [DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2-2'-azinobis(3-ethylbenzothiazoline)-6-sulphonic acid) and FRAP (Ferric Reducing Antioxidant Power)]. Among all the samples tested, phytochemical content was high in peel and seed extracts as compared to the flesh in all three species. Seed extract of *M. quadrifida* was most effective in scavenging the DPPH and ABTS free radical as well as the most effective in reducing the Fe^{3+} to Fe^{2+} as compared to other parts/species. These antioxidant activities were contributed by the high phenolic and flavonoid contents in *M. quadrifida* seed. Correlation test showed positive relationship between the phenolic and flavonoid contents with ABTS ($r = 0.563$, $r = 0.550$, $p<0.01$) and FRAP ($r = 0.896$, $r = 0.460$, $p<0.01$). As a conclusion, all parts of *Mangifera* fruits could be further utilize as natural antioxidants.

Key words: *M. caesia*, *M. quadrifida*, *M. odorata*, phytochemicals, antioxidant, vegetables

INTRODUCTION

Excessive exposure to Reactive Oxygen Species (ROS) has caused cells damage which results in generation of some chronic diseases (Ali-Hassan *et al*., 2013). Antioxidant is known as 'free radical scavenger', a compound that neutralises the reactive molecules (such as ROS) by donating electron and prevent the oxidative damage on cells (Sen *et al*., 2010). Until recently, the search for natural antioxidant in replacement to synthetic antioxidant is still on going. The natural antioxidant which possess wide range of biological and therapeutic activity could be obtained from fruits, vegetables, nuts, flowers and bark of tree (Torres-Leon *et al*., 2016). Consumption of fruits and vegetables is one of the good strategies to decrease the potential of getting various types of diseases such as cancer, cardiovascular disease, Alzheimer disease and age-related disease (Liu, 2013). Disease prevention ability of fruits is contributed by their phytochemical constituents including phenolics, vitamins and carotene that are able to prevent the oxidation of biomolecules (lipid and protein) in human body (Thaipong *et al*., 2006). Thus, many researchers have focusing on finding the bioactive compounds originated from fruits which might give health benefit to human. Even though many research have been done on fruits, the

Corresponding Author: Mohd Fadzelly Abu Bakar, Faculty of Applied Sciences and Technology (FAST), Universiti Tun Hussein Onn Malaysia (UTHM), Hub Pendidikan Tinggi Pagoh, 1 km, Jalan Panchor, Muar, 84600 Johor, Malaysia

information on underutilized fruits are still lacking (Ahmad *et al*., 2015). There are a few local fruits that are still underutilized and the benefit is not yet fully explored in Sabah, Malaysia, Borneo (Abu Bakar *et al*., 2009). Most of these underutilized fruit could be obtained from the wild or sold in small-scale market such as tamarillo (*Cyphomandra betacea*), tarap (*Artocarpus odoratissimus*) and dabai (*Canarium odontophyllum*).

Genus *Mangifera* (family Anacardiaceae) is believed to be originated from South-East Asia (Graham and Woodhead, 2011) and some species are considered as less-known and underutilized. Based on floral morphology, 69 *Mangifera* species have been identified (Kostermans and Bompard, 1993) which 27 species in this genus produce edible fruits. In Borneo, there are 10 species of *Mangifera* that could be found which includes Bambangan (*Mangifera pajang*), Mangga Wani (*M. odorata*) and Bundu (*M. caesia*). Most of the research has been focusing on popular species such as common mango (*Mangifera indica*). Previous research on different parts of *Mangifera* fruits showed that members of this genus have the potential to act as antioxidant, anti-bacterial, anti-viral and anti-cancer (Abbasi *et al*., 2017; Abu Bakar *et al*., 2009; Ajila and Rao, 2008; Guha *et al*., 1996; Kaur *et al*., 2010).

Mangifera caesia Jack locally known as Bundu, in Sabah has been categorized in subgenus Limus (Marchand) Kosterm (Litz, 2009). In other parts of Malay Archipelago, this species is locally known as Binjai and Kemang. This species is naturally distributed in Sumatera, Borneo Island and Peninsular Malaysia (Normah, 2003). *M. caesia* had been introduced to the other part of Southeast Asia such as Phillipines and Thailand. *M. caesia* usually grow at lowland tropical area and rarely found in forest. Infloresence of *M. caesia* occur between month of February and April and the fruits produced from the month of August to October. Edible matured fruits have fragrant smells, whitish flesh and sweet-sour taste with high fibre, vitamin C and protein contents (Brooke and Lau, 2013). According to Wong and Siew (1994), *M. caesia* flesh contained 65 volatile components which comprised of esters (71.3%) and alcohols (23.2%) as the two major groups and ethyl 3-methylbutanoate (40.0%) as the most abundant compound. *M. caesia* fruit has the potential to be commercialized as the fruits could be freshly eaten, make into pickles and often used to prepare chillies-based spices (Normah, 2003).

Mangifera quadrifida Jack locally known as Dumpiring and Asam Kumbang is an endemic species to Malesia (Brunei, Malaysia, Kalimantan, Sumatera and Java Island). Another *Mangifera* species, *Mangifera odorata* Griffith could be found in Malaysia, Indonesia (Sumatera, Java Island and East Kalimantan), Guam and Phillipines. *M. odorata* is locally known as Mangga Wani in Sabah, whereas in Peninsular Malaysia, it is known as Kuini (Normah, 2003). In local market, Mangga Wani has been sold at high price due to its palatable and fibreless texture, juicy and sweet taste with the absence of strong odour. *M. odorata* contain isoflavones diadzein and genistein which clustered into major phytoestrogen group. About 73 volatile compounds have been found in *M. odorata* with the main classes are oxygenated monoterpenes (45.4%) and esters (33.0%) and α-terpineol (31.9%) was the major compound. Few researchers have evaluated the polyphenolic and antioxidant activities of few *Mangifera* species including *M. caesia*, *M. odorata* and *M. quadrifida* (Sulaiman and Ooi, 2012). However, the study only focused on the edible mature green and ripe flesh part of the sample without taking into account the seed and peel parts.

Thus, current study was conducted to compare the phytochemicals content (total phenolics, total flavonoid, total carotenoid and total anthocyanin content) and antioxidant potential [1,1-diphenyl-2-picrylhydrazyl radical (DPPH), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammoniumsalt (ABTS) and Ferric Reducing Antioxidant Potential (FRAP)] of different fruit parts (flesh, seed and peel) of the *Mangifera* species (*M. caesia*, *M. odorata* and *M. quadrifida*) that could be found in Borneo.

MATERIALS AND METHODS

Plant material: Sampling method was adapted from Ali Hassan *et al*. (2013) with slight modification. Three selected wild Borneo mangoes from *Mangifera* genus were collected. The authentication process was made by Mr. Johnny Gisil (Plant Taxonomist at Universiti Malaysia, Sabah). The fruit samples were cleaned, weighed and separated into peel, flesh and seed. Each part was cut into smaller pieces, freeze-dried for three days and grind into fine powder. The fine powder was sieved to get uniform size. Air-tight container was used to keep the sample at-20°C for further analysis.

Extraction: The extraction method was adapted from Velioglu *et al*. (1998) with slight modification. About 0.1 g freeze-dried sample was added to 100 mL 80% (v/v) methanol. The mixture was shaken for 2 h at 200 rpm by using orbital shaker and filtered by using filter paper. The supernatant was decanted into vial for further analysis.

Determination of moisture content: The moisture content was determined by weighing before and after the samples being freeze-dried. The percentage of moisture content was calculated by using this formula:

$$\text{Moisture content (\%)} = ((\text{Fresh weight} - \text{dry weight})/\text{Fresh weight}) \times 100$$

Determination of total phenolic content: Total phenolic content was determined using Folin-Ciocalteu method (Velioglu *et al*., 1998). Folin-Ciocalteu reagent was prepared by diluting 10 mL of Folin-Ciocalteu with 90 mL distilled water. About 100 μL of samples extract were mixed with 0.75 mL of Folin-Ciocalteu reagent and vortexed for 15 sec. After 5 min, 0.75 mL of sodium bicarbonate (60 g/L) solution were added to the mixture and allowed to stand at room temperature for 90 min. The absorbance value was measured spectrophotometrically at 725 nm. Gallic acid was used as standard in the range of 0-100 μg/mL and the total phenolic content were expressed as gallic acid equivalent in 1 g of dried sample (mg GAE/g).

Determination of total flavonoid content: Aluminium colorimetric method was used to determine total flavonoid content (Dewanto *et al*., 2002) with slight modification. Briefly, 1 mL of extract was added to 4 mL distilled water and 0.3 mL of 5% w/v sodium nitrite in a beaker. After 6 min, 0.6 mL of 10% w/v aluminium chloride hexahydrate were added and left for 5 min. Then, 2 mL sodium hydroxide (1M) was added to the solution and the mixture was vortexed before placing it into microplate. The absorbance value was measured at 510 nm by using spectrophotometer. Catechin (20-100 μg/mL) was used as a standard. Results were compared with standard graph and represented in mg catechin equivalent/g (mg CE/g) sample.

Determination of total anthocyanin content: Total anthocyanin content was measured by using a spectrophotometric pH differential protocol (Giusti and Wrolstad, 2001) with slight modification. Briefly, 0.5 mL extract was mixed thoroughly with 3.5 mL potassium chloride buffer; pH 1.0 (0.025M). The mixture was mixed well by using vortex and allowed to stand for 15 min. The absorbance values were measured at 515 and 700 nm against distilled water blank. The extract was mixed with 3.5 mL of 0.025 M sodium acetate buffer; pH 4.5 and allowed to stand for 15 min. The absorbance value was measured at the same wavelength. The total anthocyanin content was calculated by using the following formula:

$$\text{Total anthocyanin content (mg/100 g of dried sample)} = A \times Mw \times DF \times 1000/(\varepsilon \times C)$$

Where:

A = Absorbance = $(A_{515}-A_{700})$ pH 1.0-$(A_{515}-A_{700})$ pH 4.5
Mw = Molecular weight for cyanidin-3-glucoside = 449.2
DF = Dilution Factor of the samples
ε = The molar absorptivity of cyanidin-3-glucoside = 26,900
C = The concentration of the buffer (mg/mL)

Results were expressed as mg cyanidin-3-glucoside equivalents (c-3-gE)/g of dried sample.

Determination of total carotenoid content: Carotenoid content in the extract was determined based on method described previously (Hess *et al*., 1991). About 150 μL extract with 150 μL distilled water and 300 μL methanol were mixed in a beaker. The mixture was extracted with 600 μL hexane solution and centrifuged at speed 2000×g in 5 min at 4°C. Two layer of solution were formed. The absorbance value was measured at 350 nm by using organic layer solution. β-carotene was used as a standard. Result was expressed in mg BC/100 g sample.

DPPH (2, 2-diphenyl-1-pycrylhydrazyl) radical assay: The scavenging activity of the extract was estimated by using DPPH as a free radical model (Mensor *et al*., 2001). DPPH (0.3 mM) was prepared by dissolving 0.0118 g DPPH powder into 100 mL methanol. About 1 mL from 0.3 mM DPPH methanol was added to 2.5 mL sample extract with the different concentration and allowed to stand for 30 min at room temperature in dark condition. The absorbance value was measured spectrophotometrically at 518 nm. The absorbance value of blank and control solution was taken. The percentage of scavenging activity (%) was calculated by using this formula:

$$\text{Scavenging activity (\%)} = 100-\left[\left(\frac{Abs_{sample}-Abs_{blank}}{Abs_{control}}\right)\times 100\right]$$

Where:

Abs_{blank} = 1 mL 80% (v/v) methanol+2.5 mL extract
$Abs_{control}$ = 1 mL 0.3 mM DPPH methanol+2.5 mL 80% (v/v) methanol

The percentage of ascorbic acid for all samples was plotted. The final result was expressed as an EC_{50} (Extract Concentration which able to inhibit 50% of the used DPPH amount).

FRAP (Ferric Reducing/Antioxidant Power) assay: The ability of the extract to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) was adapted from previous method (Benzie and Strain, 1996) with slight modification. FRAP reagent was prepared by adding 300 mM acetate buffer (pH 3.6) with 10 mM TPTZ and 20 mM $FeCl_3$. $6H_2O$ with ratio 10:1:1. The FRAP reagent was used as a blank and was measured at 593 nm by using spectrophotometer. About 100 μL sample extract and 300 μL distilled water were added to the blank in test tube. After 4 min, second reading was taken. Fe(II) was prepared as a standard using several concentrations from 0-100 μg/mL. A standard curve was prepared by plotting the FRAP value of each standard against its concentration. The result was expressed as the concentration of antioxidant having a ferric reducing ability in 1 g of sample (μM/g).

ABTS (2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid): ABTS decolorization assay was adapted from Re *et al.* (1999) with slight modification. Briefly, 7 mM of ABTS solution and 2.45 mM potassium persulfate were added to a beaker to prepare $ABTS^+$ radicle that produce blue-green colour. The mixture was allowed to stand for 16 h in a dark room to prevent incomplete oxidation processes. The mixture was diluted with 80% methanol in order to obtain absorbance of 0.7±0.2 units at 734 nm. Then, 300 μL extract was added to 3 mL $ABTS^+$ solution. The mixture was vortexed for 45 sec and was transferred into cuvette. The absorbance value was measured at 734 nm by using spectrophotometer. Ascorbic acid was used as standard in the concentration range 0-60 μg/mL. The final result was expressed as mg ascorbic acid equivalent antioxidant capacity in 1 g of sample (mg AEAC/g).

Statistical analysis: All experiments were carried out in three replicates in three independent experiments. The result was presented as mean±standard deviation. The data was statistically analysed by using one-way ANOVA with significance value of $p<0.05$ to test the significance difference between samples. Pearson's correlation was used to determine the relationship between phytochemicals and antioxidant activity.

RESULTS AND DISCUSSION

Phytochemical composition of peel, flesh and seed, *M. caesia*, *M. quadrifida* and *M. odorata*: The phytochemicals that were investigated in this study are Total Phenolic (TPC), Total Flavonoid (TFC), Total Anthocyanin (TAC) and Total Carotenoid (TCC) contents of peel, flesh and kernel extract of *M. caesia*, *M. quadrifida* and *M. odorata*.

Moisture content: The water content in the flesh of all *Mangifera* species was in the range of 77-91%. For peels, the water content is between 60-74% whereas in seed the water content was about 55-63%.

Total phenolic and flavonoid content: The value of TPC and TFC for all parts of fruits differed significantly ($p<0.05$) in the range from 12.11-237.22 mg GAE/g. This is due to the distribution of various types of phenolics and flavonoid on each species and fruit parts. As shown in Table 1, seed extract of *M. quadrifida* showed the highest phenolic content (237.22 mg GAE/g) whereas, *M. odorata* flesh showed the lowest total phenolics (12.11 mg GAE/g). The overall pattern of total phenolics are as follows: *M. quadrifida* seed, *M. caesia* seed, *M. odorata* seed, *M. quadrifida* peel, *M. caesia* peel, *M. quadrifida* flesh, *M. odorata* peel, *M. caesia* flesh, *M. odorata* flesh. Seeds of all *Mangifera* species exhibited higher phenolic content as compared to peel and flesh for each species. These result is in agreement with previous study by Abu Bakar *et al.* (2009) on bambangan (*M. pajang*) which showed seeds have higher TPC as compared to other fruit parts. Phenolics are able to prevent damage resulting from oxidative processes by oxygen molecules which is essential in the process of seed germination.

According to Soong and Barlow (2004), high phenolic acid such as gallic acid, ferulic acid, ellagic acid and other componds also help to contribute to most of phenolic content in seed of mangoes and longans. The TPC of *M. caesia* (18.44±0.34 mg GAE/g), *M. quadrifida* (44.89±0.73 mg GAE/g) and *M. odorata* (12.11±0.19 mg GAE) flesh in current study were found to be higher than the flesh aqueous extract of the similar species in the previous study (Sulaiman and Ooi, 2012). The differences in the TPC of similar fruits from previous study might due to the locality of sampling and solvent used for extraction which affects the phytochemical contents. *M. caesia* and *M. odorata* flesh contain significant amount of gallic acid, vanilic acid and mangiferin. Phenolic compounds that could be found in *M. quadrifida* flesh are gallic acid, protocatechuic acid, p-Hydroxybenzoic acid, vanillic acid and mangiferin (Sulaiman and Ooi, 2012). Predominant polyphenol that could be found in mangoes is mangiferin (Guha *et al.*,1996; Luo *et al.* 2012).

Generally, there are more than 8000 types of phenolic compounds that have been recognised and flavonoid is the major class in this compound. Phenolic content in *M. quadrifida* seed was 11 times of its total flavonoid content. Total Flavonoid (TFC) of the *Mangifera* hydromethanolic extract is shown in Table 1. For *M. odorata* (25.58 mg CE/g) and *M. caesia*, the peel part showed high concentration of TFC followed by

Table 1: Phytochemical contents of different parts of fruits of *M. caesia*, *M. quadrifida* and *M. odorata*

Sample	Fruit parts	Total phenolic[1]	Total flavonoid[2]	Total anthocyanin[3]	Total carotenoid[4]
M. caesia	Peel	69.11±0.91	19.42±0.22	0.23±0.11	6.30±0.93
	Flesh	18.44±0.34	10.47±0.13	0.16±0.04	9.56±0.44
	Seed	100.88±0.96	17.47±0.67	N.D	0.81±0.25
M. quadrifida	Peel	72.74±0.36	18.67±0.22	N.D	15.19±0.84
	Flesh	44.89±0.73	11.33±0.09	N.D	18.67±0.59
	Seed	237.22±2.25	20.94±0.39	N.D	10.37±0.84
M. odorata	Peel	31.06±0.48	25.58±0.08	N.D	39.11±1.02
	Flesh	12.11±0.19	9.07±0.27	N.D	52.07±2.39
	Seed	89.87±0.62	15.35±0.14	N.D	26.52±0.68

Values are presented as mean±SD (n = 3); ND = Not Detected; [1]Total phenolic content was expressed as mg gallic acid equivalents in 1 g of dried sample (mg GAE/g); [2]Total flavonoid content was expressed as mg catechin equivalents in 1 g of dried sample (mg CE/g); [3]Total anthocyanin content was expressed as mg of cyanidin-3-glucoside equivalents in 100 g of dried sample (mg c-3-gE/100 g); [4]Total carotenoid content was expressed as mg of β-Carotene in 1 g of dried sample (mg BC/100g dried sample)

the corresponding seed and flesh. However, for *M. quadrifida*, the seed part displayed the highest TFC compared to the peel and flesh. According to Abbasi *et al.* (2016), *M. indica* peel contain higher flavonoid compared to the flesh. The similar result was found by Price *et al.* (1995) when investigating the chemical compound in grapes where the exposed part of grapes (skin) consist higher phenolics compared to the unexposed part. The finding suggest that the exposed fruit part to sunlight such as peels are prone to oxidative stress which could stimulate the production of phenolic and flavonoid compounds to prevent tissues damage (Karimi *et al.*, 2013). Other than that, increasing enzyme activity in phenolic acid synthesis is driven by high intensity of light (Kumari *et al.*, 2009). Thus, phenolic and flavonoid content are higher in sun-exposed fruit parts compared to unexposed parts (Zobel and Clarke, 1999).

Total anthocyanin content: Anthocyanin content was determined by measuring the changes of absorbance on two distinct pH value which were pH 1.0 and pH 4.5 at absorbance 515 nm and 700 nm. Changes on anthocyanin chromophore structure happens at pH 1.0 and pH 4.5 where coloured oxonium form at pH 1.0 and uncolored hemiketal form at pH 4.5 (Abu Bakar *et al.*, 2009). Current result showed that anthocyanin could only be found on peel and flesh of *M. caesia* in low quantities (Table 1). All fruit parts of *M. quadrifida* and *M. odorata* did not show positive result for anthocyanin measurement. Previous study on other *Mangifera* species, *M. pajang* fruit displayed low amount of anthocyanin present on peel and flesh part (Abu Bakar *et al.*, 2009). In addition, *M. indica* peel extract has approximately 203-565 mg anthocyanin content in every 100 g sample (Ajila *et al.*, 2007). Type of species give influence on anthocyanin content. Generally, anthocyanin is the pigment that contribute to the red, pink, blue and purple colour of plants parts (Ancos *et al.*, 2000; Zia-UL-Haq *et al.*, 2014). Those colours are absent in all studied sample which could be the evidence for low quantities of anthocyanin in *Mangifera* species.

Total carotenoid content: Carotenoid is a pigment that contribute to yellow, orange and red colour which could be found on all plant organs and tissues (Howitt and Pogson, 2006). Total Carotenoid Content (TCC) was found being most abundant in *M. odorata* (52.07±2.39 mg BC/100 g in flesh, 39.11±1.02 in peel and 26.52±0.68 in seed) than *M. quadrifida* and *M. caesia*. For each species, the edible part of fruits exhibit the highest carotenoid content in comparison to peel and seed ($p<0.05$). This study is in agreement with Hassan *et al.* (2013) that showed the flesh of *Cyphomandra betaceae* flesh contain higher amount of TCC compared to the peel. According to Ajila *et al.* (2007), the range of TCC in mango varieties peel is 7.4-43.6 mg/100 g which is similar to our study that showed 6.30-52.07 mg BC/100 g TCC. The presence of carotenoid in seed is vital for the production of plant hormone such as Abscisic Acid (ABA) and seed dormancy (Maluf *et al.*, 1997). Antioxidant potential in seeds is contributed by carotenoid to control membrane damage caused by free radical (Calucci *et al.*, 2004; Pinzino *et al.*, 1999). The carotenoid is expected to contribute to the antioxidant activity of extract (Asif *et al.*, 2016).

Antioxidant activity of different parts (peel, flesh and seed) of different *Mangifera* species: The antioxidant capacity of selected *Mangifera* species was analysed by three different method namely DPPH, ABTS and FRAP assays.

DPPH radical scavenging activity: The presence of antioxidant compound in a sample could be determined through the decolourization of purple coloured DPPH radical into yellow hydrazin. EC_{50} of the concentration of sample extract indicate its ability to scavenge DPPH free radical at 50% (Karadag *et al.*, 2009). The radical scavenging activity of the extract as shown in Fig. 1-3. Current finding suggests that peel and seed of *M. caesia* (Fig. 1), peel and seed of *M. quadrifida* (Fig. 2) and seed of *M. odorata* (Fig. 3) had better scavenging activity as

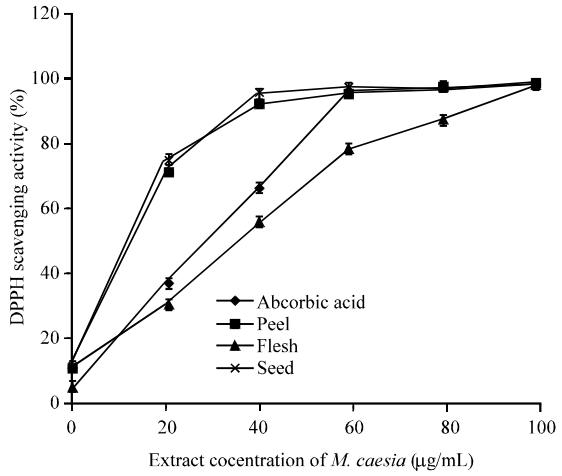

Fig. 1: The scavenging activity of 80% methanol extracts of peel, seed and flesh part of *M. caesia* assayed by DPPH free-radical scavenging method. Values are presented as mean±SD (n = 3)

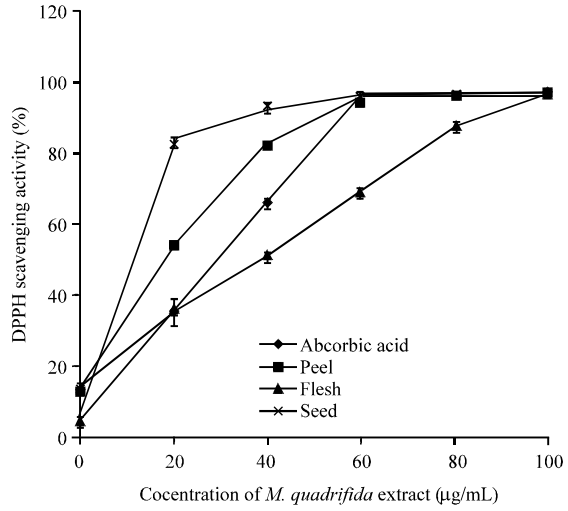

Fig. 2: The scavenging activity of 80% methanol extracts of peel, seed and flesh part of *M. quadrifida* assayed by DPPH free-radical scavenging method. Values are presented as mean±SD (n = 3)

compared to ascorbic acid (positive control). The least EC_{50} value had been shown by the seed of *M. quadrifida* and *M. odorata* which are 12.00 µg/mL. This result is in line with finding by Abu Bakar *et al.* (2009) on *M. pajang* that shows seeds are more effective in DPPH scavenging activity as compared to peel and flesh. As shown in Table 2, seed extract for all *Mangifera* species was able to scavenge 50% free radical at low concentration. Optimum concentration for *M. odorata* seed was at the concentration of 40 µg/mL whereas the optimum concentration for *M. quadrifida* seed in scavenging

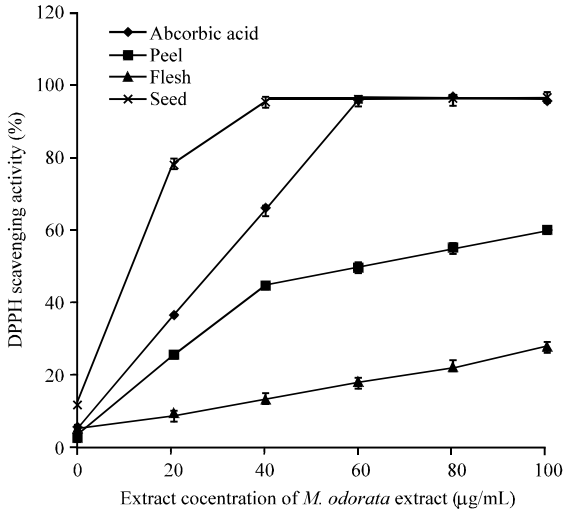

Fig. 3: The scavenging activity of 80% methanol extracts of peel, seed and flesh part of *M. odorate* assayed by DPPH free-radical scavenging method. Values are presented as mean±SD (n = 3)

Table 2: Antioxidant activity of *Mangifera* spp

Sample	Fruit parts	DPPH (EC_{50})[1]	FRAP[2]	ABTS[3]
M. caesia	Peel	12.50	165.07±0.54	101.95±0.06
	Flesh	36.00	33.08±2.24	59.29±1.22
	Seed	13.00	143.43±1.91	101.84±0.09
M. quadrifida	Peel	18.50	107.49±0.48	101.67±0.04
	Flesh	39.50	77.79±2.89	91.11±0.32
	Seed	12.00	262.47±2.28	101.99±0.02
M. odorata	Peel	64.00	66.97±1.48	78.14±0.48
	Flesh	-	12.20±0.14	8.52±0.58
	Seed	12.00	201.24±0.83	101.91±0.09
Ascorbic acid		29.40		

Values are presented as mean±SD (n = 3); [1]DPPH free radical scavenging activity represented by EC_{50} was expressed as µg/mL; [2]FRAP was expressed as µM ferric reduction to ferrous in 1g of dry sample; [3]ABTS free radical scavenging activity was expressed as mg ascorbic Acid Equivalent Antioxidant Capacity (AEAC) in 1 g of dry sample

activity was at 60 µg/mL. All samples showed significant difference ($p<0.05$) for EC_{50} value. Phenolics and flavonoid showed negative correlation with DPPH activity with value r = -0.622 and r = -0.626, respectively ($p<0.01$). Anthocyanin and carotenoid showed positive correlation with DPPH.

Ferric reducing/antioxidant power assay: FRAP method is one of method to determine antioxidant by reducing action of ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex into blue complex of Ferrous Tripyridyltriazine (Fe^{2+}-TPTZ) (Benzie and Strain, 1996). The reducing ability of *M. quadrifida* seed was the highest compared to other extract. Effective reducing capacity of Fe^{3+} ion by phenolic is related to hydroxylation and polyphenol conjugate bonding (Pulido *et al.*, 2000). The reducing ability of the

tested extract was in the order of *M. quadrifida* seed, *M. odorata* seed, *M. caesia* peel, *M. caesia* seed, *M. quadrifida* peel, *M. quadrifida* flesh, *M. odorata* peel, *M. caesia* flesh, *M. odorata* flesh. The FRAP antioxidant activity is differed significantly between species and fruit parts. Fe^{3+} reducing ability display strong positive correlation with phenolic ($p = 0.896$, $p<0.01$) and moderate correlation with flavonoid ($p = 0.460$, $p<0.01$). Present result was in agreement with Abu Bakar *et al.* (2009) and Soong and Barlow (2004). These showed that both compound have redox characteristic and capable of become effective reducing agent. In contrast, carotenoid and anthocyanin display negative correlation with FRAP activity.

ABTS assay: Another method of determining the antioxidant activity in sample is by measuring the ability of antioxidant compound to eliminate ABTS radical cation (Prior and Cao, 2000). Stable green-blue $ABTS^{+}$ solution is formed by the reaction between ABTS and potassium persulfate (Ashgar *et al*., 2008). *M. quadrifida* seed extract showed the highest scavenging activity (101.99 mg/g) through the decolorization of ABTS solution. Phenolic and flavonoid compound which are the effective electron donor might contribute to the scavenging ability of this sample. Electron or hydrogen molecule from phenolic in extract was able to reduce radical cation and this could be observed through the decolorization of solution. ABTS scavenging activity of each extract differed significantly ($p<0.05$). Analysis of correlation showed that ABTS radical scavenging activity was moderately correlated with phenolic ($r = 0.563$, $p<0.01$) and flavonoid ($r = 0.550$, $p<0.01$). Negative correlation exists between carotenoid and anthocyanin with ABTS assay which is similar to correlation with FRAP assay. While consuming the fruit, the peel and seed tend to be discarded. However, the discarded part demonstrate the major source of phytochemical and antioxidant. Hence, the by product should be consider to be developed as nutraceutical products.

CONCLUSION

As a conclusion, all *Mangifera* spesies in current study has a potential to act as natural antioxidant and may have positive impact on human health. High phytochemical content in the tested fruit extract contributed to the high antioxidant activity. Promoting this underutilized fruits to public may increase economic potential in agricultural and nutraceutical field.

ACKNOWLEDGEMENTS

The researchers would like to acknowledge the Ministry of Higher Education for financial assistance under Fundamental Research Grant Scheme (FRGS) (Grant No.: 1560). The researchers would also like to acknowledge, Institute for Tropical Biology and Conservation and Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Malaysia, for the use of the laboratory facilities and technical assistance.

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