ORIGINAL ARTICLE

Manilkara zapota (L.) P. Royen: Potential Source of Natural Antioxidants

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ABSTRACT

Introduction: *Manilkara zapota* (L.) P. Royen or sapodilla is a fruit-bearing tree that has been cultivated mainly in tropical areas including Mexico and South East Asia. The fruits and the other parts of *M. zapota* plant have been used since ages ago for various medicinal purposes. However, the data on the antioxidant properties of various parts of *M. zapota* is limited. Therefore, we aimed to measure the content and capacity of antioxidants in various *M. zapota* plant parts and also to screen the phytoconstituents present in the part with the highest antioxidant content and capacity. **Methods:** The *in vitro* antioxidant evaluation including the content of total phenolic (TPC) and total flavonoids (TFC) as well as β-carotene bleaching and 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability of the leaves, seeds, flesh, and peels of *M. zapota* extracted by aqueous and ethanol were determined. The plant part that exhibited the highest TPC, TFC, and antioxidant capacity was selected for phytoconstituents identification using liquid chromatography mass spectrometry. **Results:** *M. zapota* leaves aqueous extract exhibited the highest TPC, TFC, and antioxidant capacities and therefore selected for phytoconstituents identification. Our study provide additional data in which a total of 39 phytoconstituents have been identified in the *M. zapota* leaves including m-coumaric acid, quinic acid, robinetinidol-4alpha-ol, isoorientin 6''-O-caffeate, apocynin A, and C16 Sphinganine. **Conclusion:** Thus, our study revealed that *M. zapota* leaves aqueous extract has potential as a promising naturally-occurring antioxidant candidate which could be useful for medicinal and nutritional functions.

Keywords: Antioxidant, Flavonoids, Leaves, *Manilkara zapota*, Phenolic, Phytoconstituents

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INTRODUCTION

Plants are rich in phytochemicals as well as dietary nutrients. They are also being used as a source of medication since ages ago despite the great advances of modern medicine. The plant-based diet is said to be effective in reducing the possibility of chronic disease occurrence such as type 2 diabetes (1), cardiovascular diseases (2), as well as various cancers (3). These illnesses are the consequences of lipid, proteins, and/or deoxyribonucleic acid (DNA) damage from oxidative stress caused by excessive free radical production in the biological system (4). In addition to the internal antioxidant system which already exists in the human body, an external source of antioxidant especially the naturally occurring ones could be of great importance in combating oxidative stress by neutralizing the free radicals. There are numerous phytochemicals present in plants including phenolics, flavonoids, terpenoids, saponins, tannins, glycosides, and alkaloids (5). The antioxidant properties of plants were mostly attributed to the existence of phenolic compounds (6,7). They are plant secondary metabolites that comprise aromatic rings with hydroxyl groups. They neutralise free radicals by donating either electron or hydrogen atom to the free radical, as well as chelating metal ions. The largest yet most important groups of phytochemicals found in plants are of phenolic acids and flavonoids.

Manilkara zapota (L.) P. Royen, a lowland rainforest species is known as ciku or sapodilla in Malaysia and other countries, respectively. It is mainly cultivated for their sweet fruits as refreshments, their woods used for making furniture, and the latex was collected for chewing gum and gloves production (8). Apart from

that, the fruits and other underutilized parts of *M. zapota* such as seeds and leaves were also used traditionally for medicinal purposes. For instance, the fruits were used to prevent diarrhea in Brunei Darussalam (9). Other than that, the fruits and crushed seeds were used to prevent diuretic-related oedema and kidney stone formation while the leaves decoction was consumed to treat fever, haemorrhage, wounds, and ulcers by folks in India (10). Their medicinal properties have been scientifically studied to clarify the traditional uses of *M. zapota* parts. Previous studies have reported that the flesh exhibited high antioxidant (11), anti-diabetic, and anti-lipidemic activity (12). The peels were reported to exert antioxidant (13) and antibacterial properties (14). The seeds were considered effective in inhibiting hypoglycaemia (15), lowering the blood glucose level in diabetic mice (16), and also exerted significant antibacterial properties (17). Besides, the leaves were also revealed for their outstanding medicinal properties including significant antioxidant, antimicrobial, anti-inflammatory (18), anti-tumour (19), anti-cancer against various cancer cell lines (20,21), anti-pyretic (22), anti-diarrhoeal and anti-nociceptive (23), and urate-lowering properties (24).

However, the data on antioxidant properties of various parts of *M. zapota* extracted by aqueous and ethanol is limited. Hence, we aimed to measure and compare the content and capacity of antioxidants in various *M. zapota* plant parts and also to screen the phytoconstituents present in the part with the highest antioxidant content and capacity.

MATERIALS AND METHODS

Plant sampling and verification

The matured leaves (dark green) and fruits of *M. zapota* were collected in September 2018 from the plant growing in a home garden at Kuala Pilah, Negeri Sembilan, Malaysia. The plant's species confirmation was conducted at Biodiversity Unit, Institute of Bioscience, Universiti Putra Malaysia. The specimens were verified and given the voucher number of MFI 0036/19. The *M. zapota* plant parts including the fruits (flesh and peels), seeds and leaves were used in this study (Figure 1).

Sample preparation

Briefly, *M. zapota*'s leaves, seeds, flesh, as well as peels were manually separated and washed using running tap water before a drying process using a ventilated dryer (Protech, Malaysia) at 35 °C for 72 h before grinding into powdered form (25). The different parts of *M. zapota* were extracted according to Wan et al. with some adjustment (26). About 50 g of the powdered samples were soaked in 500 mL of distilled water (aqueous) or 70 % (v/v) ethanol (Merck, Darmstadt, Germany) and agitated on an orbital shaker (Wiseshake, Lindau, Switzerland) at 100 rpm at room condition overnight. Then, the suspension was filtered by a vacuum pump and filter paper (Sigma Aldrich, Darmstadt, Germany)

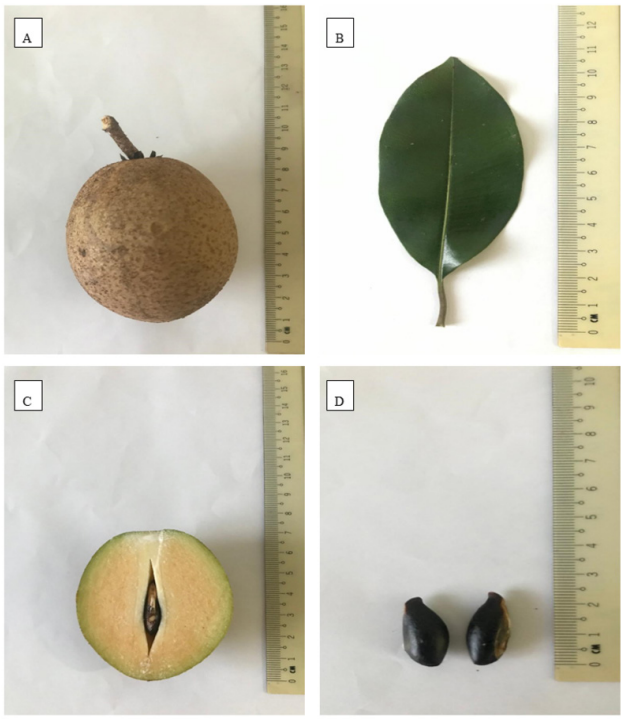

Figure 1: Manilkara zapota plant parts. The fruit with peel (A), leaf (B), flesh (C), and seeds (D).

and the remaining were then re-extracted. Lastly, the filtrates from aqueous extracts were dried by a benchtop freeze dryer (VirTis, Pennsylvania, USA) to obtain a concentrated powder, while the filtrates from 70% ethanol extracts were collected by a rotary evaporator (Buchi, Flawil, Switzerland) at the temperature of 48 °C. The percentage of yield for each extracts in both solvents as shown in Table I was based on the weight of dried and ground plant materials.

Table I: The weight of starting materials and the percentage of extraction yield of M. zapota parts in different solvents.

	Initial weight (g)	Percentage of yield (%)
70% Ethanol		
Leaves	50.00	11.24
Peels	50.00	38.50
Flesh	50.00	79.82
Seeds	15.00	19.20
Water		
Leaves	46.00	7.98
Peels	15.00	29.97
Flesh	50.00	24.20
Seeds	33.00	8.53

Determination of total phenolic content

Total phenolic content (TPC) of plant samples were analysed using Folin-ciocalteu chemical agent (Merck, Darmstadt, Germany) following a modified protocol by Lee et al. (27). The extracts or standard (20 µL) and 100 µL of Folin-ciocalteu were pipetted into the microplate and kept at room temperature for 5 min. The sodium carbonate (Na2CO3) (Merck (Darmstadt, Germany)

solution (7.5 % (w/v)) was then added, covered, and incubated in the dark for 30 min. Lastly, the absorbance was read at 725 nm using a microplate reader (BMG Labtech, Ortenberg, Germany). Gallic acid (Sigma Chemical Co., St. Louis, MO, USA) was used as a reference and the calibration graph was plotted. The TPC were expressed as microgram (µg) of gallic acid equivalent (GAE) per 100 g of extract according to this formula:

$$TPC_{sample}\ (\text{for } 100g) = \frac{TPC_{sample}\ (\text{per mL}) \times \text{dilution factor} \times \text{voume}_{sample}}{\text{Weight}_{sample}} \times 100$$

Measurement of total flavonoid content

The *M. zapota* parts TFC was assessed using a colourimetric method of aluminium chloride with slight modification (28). Briefly, the extracts or standard solutions (20 µL) were pipetted into a microplate. Then, 6 µL of sodium nitrate (NaNO2) (Merck, Darmstadt, Germany) and 48 µL of distilled water were added and kept at room temperature for 5 min. Subsequently, 6 µL of aluminium chloride (AlCl3) (Sigma Chemical Co., St. Louis, MO, USA) was added and incubated at room temperature for 5 min. Sodium hydroxide (NaOH) (Merck, Darmstadt, Germany) (50 µL) and distilled water (80 µL) were added into a microplate and incubated at room temperature for another 15 min. Lastly, the absorbance was read at 415 nm . Catechin (Fisher Scientific, Loughborough, UK) was used as a standard. The TFC was expressed as microgram (µg) of catechin Equivalent (CE) per 100 g extract through a calibration curve of catechin using the formula:

$$TFC_{sample}\ (\text{for } 100g) = \frac{TFC_{sample}\ (\text{per mL}) \times \text{dilution factor} \times \text{voume}_{sample}}{\text{Weight}_{sample}} \times 100$$

Evaluation of antioxidant activity by β-carotene bleaching test

The ability of *M. zapota* parts to bleach β-carotene was measured following a method of Woo et al. with slight modifications (29). The working solution of β-carotene (Sigma Chemical Co., St. Louis, MO, USA) was prepared into 2 mg/mL in chloroform (R&M Marketing, Essex, UK). Then, 1 mL of working β-carotene solution was mixed with 20 µL of linoleic acid (Sigma Chemical Co., St. Louis, MO, USA) and 200 µL of Tween 20 (Sigma Chemical Co., St. Louis, MO, USA). The chloroform was then abstracted by a rotary evaporator at 30 °C for 1 min. After that, 50 mL of distilled water was added and shaken vigorously to form an emulsion. The extracts or standard butylated hydroxytoluene (BHT) (Sigma Chemical Co., St. Louis, MO, USA) (20 µL) were added into microplate. Then, β-carotene emulsion (200 µL) was added and the absorbance was read at 470 nm by microplate reader for 2 h at 50 °C with 20 min intervals. Water (aqueous) and 70 % of ethanol were used as blank for this assay.

Measurement of antioxidant capacity by DPPH radical scavenging assay

M. zapota parts' ability to scavenge free radicals was measured using modified method of 2-diphenyl-1-picrylhydrazyl (DPPH) assay described by Jing et al. (30). The DPPH working solution was prepared by mixing DPPH (Sigma Chemical Co., St. Louis, MO, USA) with methanol (Fisher Scientific, Loughborough, UK). Then, 100 µL of various concentrations of extracts and 100 µL of DPPH solution were added in the microplate. The mixture was kept in the dark at room temperature for 30 min. Then, the absorbance was read at 517 nm. At least three independent tests were performed for each extract of *M. zapota*. BHT was used as reference in this test. The DPPH radical scavenging ability of *M. zapota* parts were calculated following this formula:

$$\text{DPPH radical scavenging ability } (\%) = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

Phytoconstituent identification of *M. zapota* leaves aqueous extract by LC-QTOF MS

Liquid chromatography quadrupole time-of-flight mass spectrometry (LC Q-TOF MS) analysis was used for phytoconstituents identification of *M. zapota* leaves aqueous extract according to He et al. (31). The sample was dissolved in water and filtered using a nylon membrane filter with a pore size of 0.22 µm before analysis. The LC-QTOF MS analysis was run using Agilent 1290 Infinity LC system and chromatography was hyphenated with Agilent 6520 Accurate Mass Q-TOF mass spectrometer. The system was fitted with dual electrospray ionisation (ESI) source (Agilent Technologies, Santa Clara, CA, USA). An Agilent Zorbax Eclipse XDB-C18, Narrow-Bore 2.1 x 150 mm column was used. While the mobile phases of 0.1 % (v/v) of formic acid in water and 0.1 % (v/v) of formic acid in acetonitrile were used for phytoconstituents identification. ESI-Q-TOF MS analysis was run in both negative and positive ionisation modes. A fragmentor voltage of 125 V, 300 °C drying gas temperature, 10 L/min drying gas flow, and 10 µL sample was injected from the auto-sampler which was thermostated at 5 °C. The identification of phytoconstituents present in the sample was carried out by comparing the retention time and mass spectra with literature and database.

Statistical analysis

The data are presented as mean ± standard deviation (SD). One-way ANOVA and Tukey's test were used for pairwise comparisons. Pearson correlation test was performed to determine the relationship between TPC, TFC, and antioxidant capacities. The data were analysed using IBM Statistical Package for Social Science (SPSS) version 19.0 (SPSS Inc., Chicago, IL, USA). P-value less 0.05 was considered significant in difference.

RESULTS

Total phenolic contents of *M. zapota* extracts

The TPC of aqueous and 70% ethanol of four different parts of *M. zapota* extracts were tabulated in Table II. The highest TPC among aqueous extracts was found in the leaves ($p < 0.05$) followed by the seeds, peels, and flesh with the values ranged between 31.35 ± 0.45 and 2.86

Table II: Total phenolic and total flavonoid contents of different part of *M. zapota* extracts

Plant part	Total phenolic content (µg GAE/100 g)		Total flavonoid content (µg CE/100 g)	
	Aqueous extract	70% ethanol extract	Aqueous extract	70% ethanol extract
Flesh	2.86 ± 0.21[a]	0.73 ± 0.1[a]	1.02 ± 0.64[a]	0.08 ± 0.02[a]
Peel	2.97 ± 0.10[a]	1.23 ± 0.06[a]	1.43 ± 0.47[a]	0.16 ± 0.04[a]
Leaf	31.35 ± 0.45[b]	14.15 ± 0.48[b]	33.71 ± 8.02[b]	12.10 ± 3.12[b]
Seed	9.39 ± 1.96[c]	2.14 ± 0.02[c]	4.61 ± 2.88[a]	3.13 ± 0.03[a]

All values are expressed as mean ± SD .[a] Values with different superscript letters in the same column indicate significant difference at $p < 0.05$. GAE, gallic acid equivalents; CE, catechin equivalents

± 0.21 µg GAE/100g. A similar trend was found in the TPC of ethanol extracts where the highest TPC exhibited by the leaf part ($p < 0.05$) followed by the seeds, peels, and flesh with the values ranged from 14.15 ± 0.48 to 0.73 ± 0.01 µg GAE/100g.

Total flavonoid contents of *M. zapota* extracts

Total flavonoids content determined among leaves, seeds, flesh, and peels of *M. zapota* aqueous extracts were ranged from 33.71 ± 8.02 to 1.02 ± 0.64 µg CE/100g extract with the highest TFC exhibited by the leaves ($p < 0.05$). TFC among *M. zapota* parts of ethanol extracts however ranged between 12.10 ± 3.12 to 0.08 ± 0.10 µg CE/100g where the leaves showed the highest TFC compared to other parts ($p < 0.05$) (Table II).

Antioxidant activity of *M. zapota* parts by β-carotene bleaching test

The first assay performed to determine the antioxidant capacity of various parts of *M. zapota* was β-carotene bleaching assay. The working concentration of the extracts used for this test were 250 µg/mL. The results were summarised in Table III. The highest inhibition of β-carotene bleaching ($p < 0.05$) among aqueous extract of *M. zapota* parts was found in the leaves (49.94 ± 10.60 %) followed by seeds (47.17 ± 2.24 %) and peel (39.23 ± 11.54 %). However, among ethanol extracts only the leaves exhibited the highest antioxidant activity (48.59 ± 9.52 %) compared to other parts of the plant ($p < 0.05$). Miraculously, the leaves aqueous extract had a greater antioxidant capacity when compared to standard BHT (43.49 ± 2.64 %) ($p > 0.05$).

Table III: Antioxidant capacities of different part of *M. zapota* extracts determined using β-carotene bleaching and DPPH assays

Plant part	β-carotene bleaching (% of inhibition)		DPPH (% of scavenging activity)	
	Aqueous extract	70% ethanol extract	Aqueous extract	70% ethanol extract
Flesh	18.55 ± 6.07[a]	8.38 ± 4.11[a]	91.99 ± 1.20[a]	78.21 ± 0.04[a]
Peel	39.23 ± 11.54[ab]	13.00 ± 2.81[a]	82.04 ± 4.81[b]	91.98 ± 0.71[b]
Leaf	49.94 ± 10.60[b]	48.59 ± 9.52[b]	93.61 ± 0.59[a]	92.96 ± 0.06[b]
Seed	47.17 ± 2.24[b]	21.41 ± 7.25[a]	62.97 ± 0.20[c]	49.36 ± 0.92[c]

All values are expressed as mean ± SD [a] Values with different superscript letters in the same column indicate significant difference by Tukey's test ($p < 0.05$). DPPH, 2, 2-diphenyl-1-picrylhydrazyl. The working concentration of extracts used for both assays was 250 µg/mL.

Antioxidant capacity of *M. zapota* parts by DPPH assay

The antioxidant capacity of *M. zapota* extracts was also estimated by DPPH assay. The ability of aqueous and ethanol extracts of *M. zapota* parts in scavenging DPPH radicals was tabulated in Table III. The values were within the range of 93.61 ± 0.59 % and 62.97 ± 0.21 % where the highest value was exhibited by the leaf followed by flesh extract ($p < 0.05$). Whereas, among ethanol extracts the DPPH radical scavenging ability ranged between 92.96 ± 0.06 % and 49.36 ± 0.92 % where the greatest value showed by leaf extract ($p < 0.05$). The DPPH radical scavenging ability of aqueous and ethanol leaves extracts were higher than BHT (90.02 ± 0.73 %) ($p > 0.05$). The working concentration of the extracts used for this test were 250 µg/mL.

Correlation between TPC and TFC with antioxidant capacities

The correlation between TPC, TFC, β-carotene bleaching ability, and DPPH radical scavenging ability of *M. zapota* were analysed using the Pearson correlation test and the correlation coefficient (r) values were presented in Table IV. Based on the data, β-carotene bleaching was shown to have strong positive correlation with TPC ($r = 0.689$) and TFC ($r = 0.604$) ($p < 0.05$). Nonetheless, there were moderate negative correlation between the ability of DPPH radical scavenging and TPC ($r = - 0.533$) and TFC ($r = - 0.409$).

Table IV: Pearson correlation analyses of total phenolic content and total flavonoid content with antioxidant capacities in *M. zapota* extracts

Assay	*r* - value	
	BCB	DPPH
TPC	0.689**	-0.532*
TFC	0.604**	-0.409

** Correlation is significant at $p < 0.01$ level; * Correlation is significant at $p < 0.05$. TPC, total phenolic content; TFC, total flavonoids contents; BCB, β-carotene bleaching assay; DPPH, 2, 2-Diphenyl-1-picrylhydrazyl.

Phytoconstituents identification of *M. zapota* leaves aqueous extract by LC-QTOF MS

Based on the present findings obtained from the TPC, TFC, β-carotene bleaching, and DPPH radical scavenging assays of four different parts of *M. zapota*, the aqueous extract of the leaves has been selected to undergo phytoconstituents screening process due to its high antioxidant content and capacities. LC-QTOF MS was chosen for the identification analysis due to its high accuracy, sensitivity, and resolution to detect fragment ions. Phytoconstituents identification using this method not only helps to confirm known compounds but also give the structure of unknown compounds. Data were processed using Agilent MassHunter Qualitative Analysis B.05.00. Figure 2A and 2B illustrate the total ion chromatogram for *M. zapota* leaves aqueous extract by ESI-Q-TOF MS in both positive and negative ionisation modes, respectively. A total of 39 metabolites of various structural classes were identified in *M. zapota*

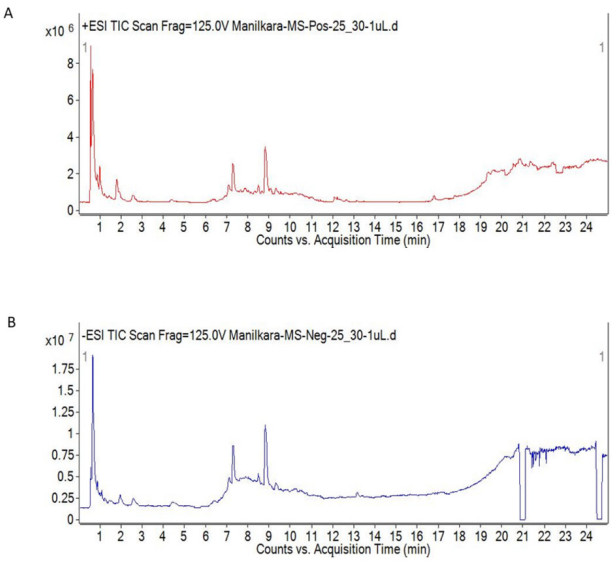

Figure 2: Mass spectrometry total ions chromatography of *Manilkara zapota* leaf aqueous extract in positive (A) and negative (B) ionisation modes.

leaf aqueous extract by comparison of their retention time and mass spectra with standards or literature data including m-coumaric acid, quinic acid, robinetinidol-4alpha-ol, isoorientin 6''-O-caffeate, apocynin A, and C16 sphinganine (Table V).

DISCUSSION

Traditionally, some of *M. zapota* parts were crushed and decocted in water and use as a traditional medicine to treat illnesses. A toxicity study conducted by Fayek et al. have reported that the median lethal dose (LD50) of *M. zapota* leaves of both aqueous and ethanol extracts in male albino mice is 80 g/kg body weight (b.w). It shows that aqueous and ethanol extracts are safe and could be administered up to 80 kg/g b.w in animals especially for long term administration (32). The in vitro study of Tan et al. also shown that methanol extract of *M. zapota* leaves was not cytotoxic to BALB/c 3T3 normal cells. Therefore, in the present study, the extraction of the leaves, seeds, flesh, and peels of *M. zapota* was done using aqueous and ethanol.

The presence of phenolic compounds in the sample can be detected by the reduction of the heteropolyphosphotungsates-molybdates mixture of Folin-ciocalteu reagent under a basic condition that leads to the blue coloured chromogen production (34). The TPC of *M. zapota* in this present study demonstrated that the aqueous extract has higher TPC than ethanol extracts. This might be due to polar compounds such as phenolic acids and flavonoids that were highly soluble in water. Comparison between parts of the plant, leaves showed the highest TPC. Our finding was consistent with a previous study of Garcia et al. where *M. zapota* leaves aqueous extract exhibited the highest TPC value of 1738.47 ± 5.52 ug GAE/mL compared to other plants leaves extracts (35).

As for the presence of total flavonoid content (TFC) in the plant parts, it was assessed via aluminium chloride colorimetric method by acid complexes production with keto and/or hydroxyl functional groups of flavonols, flavones, and flavonoids (36). The present study revealed that the highest TFC of all four parts of *M. zapota* in both solvents was also exhibited by the leaves aqueous extract. Previous study done by Chanda and Nagani found that the highest TFC value of *M. zapota* leaves was found in ethyl acetate extract (91.60 ± 0.56 mg/g) and followed by aqueous extract with the value of 19.14 ± 0.17 mg/g (37).

Slight differences on the value of TFC and TPC might be attributed to the difference of soil type and habitat. Therefore, the result cannot be generalised to other research. Other than TPC and TFC, the antioxidant activity of *M. zapota* parts were evaluated by β-carotene bleaching assay, in which the principle is based on the discolouration of β-carotene from yellow to colourless due to the free radical's production produced following linoleic acid oxidation. The β-carotene bleaching activity was detected by the presence of antioxidants that neutralised the radicals. The present study revealed that leaves aqueous extract possessed the greatest antioxidant capacity than other parts as evaluated by β-carotene bleaching activity. Besides, DPPH assay was also performed to evaluate the ability of the phytoconstituents present in the samples to act as hydrogen donors or free-radicals neutralisers. Result shows that the highest DPPH radical scavenging ability of *M. zapota* exhibited by the leaves aqueous extract.

To clarify the effect of phenolics and flavonoids on antioxidant capacities, the correlation between TPC, TFC, and antioxidant capacities was measured. Our results on the TPC and TFC of various parts of *M. zapota* showed a strong positive correlation with antioxidant capacity by β-carotene bleaching assay. These results propose that the phenolics and flavonoids in the extracts support the significant antioxidant capacity of various parts of *M. zapota* and could be used as an important indicator to choose samples for drug development or functional food ingredients.

Nevertheless, our findings on the TPC shown to have a negative correlation with DPPH radical scavenging ability whilst TFC did not. This was probably because of reducing agent, protein, genetic, agronomic and environmental factors. Some antioxidant compounds in the plant may be acted as reducing agents which unable to neutralise DPPH free radicals efficiently due to impediment. Other than that, there might be an error introduced in the assay used to determine the extracts' ability to neutralise DPPH free radical by measuring the reaction of by-products instead of the antioxidant

Table V: Tentative identification of *M. zapota* leaf aqueous extract by liquid chromatography quadrupole time-of-flight mass spectrometry (LC Q-TOF MS) analysis in negative and positive modes

No.	T_R (min)	ESI – (m/z)	ESI+ (m/z)	Tentative identification	Formula	Mass	Abundance (x 10^5)
1	0.642		455.12	unknown	$C_{13}H_{30}N_2O_9S_3$	454.58	0.509
2	0.672		268.09	S-Ribosyl-L-homocysteine	$C_9H_{17}NO_6S$	267.08	2.309
3	0.674		193.07	Quinic acid	$C_7H_{12}O_6$	192.06	6.801
4	0.674	223.05		unknown	$C_5H_{12}N_4O_4S$	224.06	8.351
5	0.716		340.12	unknown	$C_{12}H_{21}NO_{10}$	339.12	0.830
6	0.794		332.13	5′-O-beta-D-Glucosylpyridoxine	$C_{14}H_{21}NO_8$	331.13	2.632
7	0.840		229.15	Pro Leu	$C_{11}H_{20}N_2O_3$	228.15	1.581
8	0.844	479.11		unknown	$C_{16}H_{24}N_4O_{11}S$	480.12	1.457
9	0.995		182.08	m-Coumaric acid	$C_9H_8O_3$	164.05	9.950
10	1.226		593.13	Protoleucomelone	$C_{30}H_{24}O_{13}$	592.12	0.861
11	1.554	169.01		2,4,6-trihydroxybenzoic acid	$C_7H_6O_5$	170.02	1.634
12	1.805	164.07		4-(3-Pyridyl)-butanoic acid	$C_9H_{11}NO_2$	165.08	0.975
13	2.002	381.01		unknown	$C_{13}H_{10}N_4O_8S$	382.02	1.965
14	2.575		611.14	Isoorientin 6′′-O-caffeate	$C_{30}H_{26}O_{14}$	610.13	2.337
15	4.454	365.02		Unknown	$C_{13}H_{10}N_4O7S$	366.03	5.161
16	7.089		307.08	Robinetinidol-4alpha-ol	$C_{15}H_{14}O_7$	306.07	2.816
17	7.137		595.15	Kaempferol 3-(2′′-(Z)-p-coumaroyl glucoside)	$C_{30}H_{26}O_{13}$	594.14	0.880
18	7.651	447.15		Glu Asp Trp	$C_{20}H_{24}N_4O_8$	448.16	1.741
19	7.653		420.19	Gln Ser Trp	$C_{19}H_{25}N_5O_6$	419.18	0.855
20	7.733	343.07		1-Caffeoyl-beta-D-glucose	$C_{15}H_{18}O_9$	342.10	2.823
21	7.774		579.15	Apigenin 7-(2′′-E-p-coumaroylglucoside)	$C_{30}H_{26}O_{12}$	578.14	0.690
22	7.829		600.27	unknown	$C_{28}H_{41}NO_{13}$	599.26	0.652
23	8.016	289.07		Epifisetinidol-4alpha-ol	$C_{15}H_{14}O_6$	290.08	2.398
24	8.017		291.09	ent-Fisetinidol-4beta-ol	$C_{15}H_{14}O_6$	290.08	1.338
25	8.427	513.13		unknown	$C_{20}H_{26}N_4O_{10}S$	514.14	1.217
26	8.495		507.11	Quercetin 3-(6′′-acetylglucoside)	$C_{23}H_{22}O_{13}$	506.11	0.863
27	8.639		417.18	Phenylethyl primeveroside	$C_{19}H_{28}O_{10}$	416.17	0.439
28	8.811		611.16	Robinetin 3-rutinoside	$C_{27}H_{30}O_{16}$	610.15	0.488
29	8.906		371.20	5-Megastigmen-7-yne-3,9-diol 9-glucoside	$C_{19}H_{30}O_7$	370.20	1.055
30	9.029	415.20		Ethyl 7-epi-12-hydroxyjasmonate glucoside	$C_{20}H_{32}O_9$	416.21	1.281
31	9.372	467.10		Apocynin A	$C_{24}H_{20}O_{10}$	468.11	1.308
32	9.522	187.10		Nonic Acid	$C_9H_{16}O_4$	188.11	1.602
33	10.465	507.21		6-O-Oleuropeoylsucrose	$C_{22}H_{36}O_{13}$	508.22	0.946
34	12.069		274.27	C16 Sphinganine	$C_{16}H_{35}NO_2$	273.27	2.472
35	12.117		230.25	Xestoaminol C	$C_{14}H_{31}NO$	229.24	0.765
36	12.221		290.27	unknown	$C_{16}H_{35}NO_3$	289.26	1.589
37	16.792		205.05	3-Butylidene-7-hydroxyphthalide	$C_{12}H_{12}O_3$	204.08	0.475
38	17.777		403.23	Val Trp Val	$C_{21}H_{30}N_4O_4$	402.23	0.523
39	20.846		284.30	Stearamide	$C_{18}H_{37}NO$	283.29	1.738

T_R, retention time; ESI -, negative electrospray ionisation mode; ESI +, positive electrospray ionisation mode; m/z, mass-to-charge ratio

compounds that are naturally present in the extracts (38).

In general, findings from this study showed that *M. zapota* leaves possessed the greatest TPC, TFC, and antioxidant capacity compared to other parts of the plant. This could be due to higher exposure of the leaves to ultraviolet (UV) radiation from the sunlight than other parts of the plant. Plant has a natural ability to biosynthesis several of non-enzymatic antioxidant including phenolics and flavonoids that are capable of attenuating oxidative damage caused by reactive oxygen species (ROS) as they defend the system to avoid the deleterious effects of free radicals. Moreover, a higher proportion of flavonoids have been found in plants growing in tropical than those growing in cold conditions due to high exposure to UV light radiation (39).

Phytoconstituents identification is one of the methods that has been used to screen the phytoconstituents present in the plants which might contribute to the high antioxidant capacity of plant materials. In the present study, LC-QTOF-MS was used to screen the phytoconstituents present in aqueous extract of *M. zapota* leaves due to its high sensitivity, reliability, and accuracy. Aqueous extract of the leaves was chosen due to its high TFC, TPC, and antioxidant activity. From the analysis, a total of 39 phytoconstituents were identified in *M. zapota* leaves aqueous extract with nine of them were unknown compounds. There were various types of metabolites identified including phenolic compounds, amino acids, sugars, and others. The presence of m-coumaric acid, quinic acid, robinetinidol-4alpha-ol, isoorientin 6''-O-caffeate, apocynin A, and C16 Sphinganine could be the contributor to the high phenolic and antioxidant capacities in the extract. m-coumaric acid was reported to have a protective effect on hyperglycaemia in diabetic rats (40). Quinic acid, however, has been suggested to act as a radio-protective compound which has reduced the x-ray induced DNA damage (41). Other than that, Isoorientin 6''-O-caffeate was found to act as a potent antioxidant and alpha-glucosidase inhibitor (42). Apocynin A was reported to act as a nicotinamide adenine dinucleotide phosphate (NADPH) oxidase inhibitor (43). Robinetinidol-4alpha-ol, Epifisetinidol-4alpha-ol, and ent-Fisetinidol-4beta-ol are flavanols in which robinetinidol and fisetinidol were the major constituents isolated from Acacia have been reported to exhibit alpha-amylase and lipase activities (44). C16 Sphinganine could also be the contributor to antioxidant activities where an endogenous sphinganine was found to inhibit the transport of cholesterol in Niemann-Pick type C disease (45).

Our results provide clear and substantial evidence that *M. zapota* especially the leaves offer a great potential source of antioxidant against oxidative stress-related diseases. This is due to the findings of this study demonstrated that *M. zapota* leaves aqueous extract possesses high amounts of various types of phenolic compounds which might contribute to an important role in disease prevention or inhibition. These results also unveil the broad range of compounds existed in *M. zapota* leaves which could be responsible for their health benefits. However further investigation on the isolation of active compounds in the leaves extract should be carried out and validated in vivo before clinical use.

CONCLUSION

Our study provide additional data in which a total of 39 phytoconstituents have been identified in the *M. zapota* leaves aqueous extract that could be the contributors to their high antioxidant contents and capacities. Therefore, our study suggests that *M. zapota* leaves is a good source naturally occurring antioxidants.

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