International Food Research Journal 28(2): 366 - 376 (April 2021)
Journal homepage: *http://www.ifrj.upm.edu.my*

In vitro antidiabetic property and phytochemical profiling of *Mangifera odorata* pulp using UHPLC-ESI-Orbitrap-MS/MS

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Article history

Received: 4 February 2020
Received in revised form: 3 September 2020
Accepted: 2 November 2020

Keywords

vitamin, minerals, α-amylase, mangiferin, underutilised fruit

Abstract

Mangifera odorata, or known as *"kuini"* in Malay, is considered an underutilised climacteric tropical fruit. Recent studies had suggested that the by-products of *M. odorata* may serve as functional food ingredients based on their nutritional values and biological activities. Thus, the present work was aimed to determine the nutritional composition, antidiabetic properties through α-glucosidase and α-amylase inhibition assays, and phytochemical profiling of *M. odorata* pulp using ultra-high-performance liquid chromatography-electrospray ionisation orbitrap tandem mass spectrometry (UHPLC-ESI-Orbitrap-MS/MS). Proximate analyses found that *M. odorata* pulp contained high amounts of soluble fibre and moisture. The main sugar in the pulp was identified as sucrose, while ß-carotene, potassium, calcium, iron, and zinc were the major vitamins and minerals. The extraction of the sample in water, acetone, methanol, and ethanol were performed for the determination of antidiabetic assay. The results showed that 60% of ethanolic extract had the highest α-amylase inhibitory activity among the four extraction solvents. The findings revealed high levels of mangiferin in the active extract, which may be responsible for the α-amylase inhibition activity, thus contributing to lower post-prandial blood glucose. The present work provides evidence on the nutritional benefits of *M. odorata* pulp, as well as the potential use of its ethanolic extract as an α-amylase inhibitor.

Introduction

Mangifera odorata, a species of mango, is one of the underutilised climacteric tropical fruits commonly found in Peninsular Malaysia, Sabah, Singapore, Sumatera, and Java (Lim, 2012). *M. odorata* is a crossbreed of two species which are *M. indica* and *M. foetida*. *M. odorata* is known as *"kuini"* by the locals due to its strong smell and flavour. *M. odorata*, which is widely cultivated in the wet climate areas, provides a source of income to the locals (Teo *et al.,* 2002). The pulp of *M. odorata* has a fibrous texture, and when ripe, the pulp is orange in colour and juicy with a turpentine-like taste. The pulp of *M. odorata* is loaded with nutrients such as protein, calcium, and carotenoids as compared to other *Mangifera* species (Mirfat *et al.,* 2015). Besides, it also contains higher isoflavones and total phenolic content (TPC) than *M. pajang* (*bambangan*) and *M. foetida* (Ikram *et al.,* 2009), while possessing excellent antioxidant activity (Lasano *et al.,* 2019a). Additionally, the by-products of *M. odorata*, particularly the seed kernel, has been reported to possess high amounts of essential nutrients, and exhibited substantial α-amylase and α-glucosidase inhibitory properties (Lasano *et al.,* 2019b).

In the past decades, diabetes mellitus has become a major public health concern globally due to its increasing prevalence in adults from 4.7% in 1980 to 8.5% in 2014, with a projection to exceed 500 million in 2018 (WHO, 2020). Similarly, in Malaysia, the rising trend of diabetes mellitus cases is evident in the past two decades, whereby the prevalence has nearly tripled since 1996 (IPH, 2015). To date, there is no absolute preventive or curative method for diabetic patients. Hence, the antioxidant approach will be an alternative way for prevention and management of diabetes mellitus. Antioxidants mainly comprise of both enzymes and non-enzymes, which include glutathione peroxidase, catalase, vitamins, and polyphenols. Plants, especially tropical fruits, have been identified as important sources of polyphenols (Sagrin *et al.,* 2019; Gülçin, 2020). In fact, several medicinal drugs are derived from active ingredients in plants (Fabricant and Farnsworth, 2001). Apart from that, natural products of plants have none or little side effects as compared to synthetic drugs.

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Different parts of plants contain varying quantities of bioactive compounds, which possess different chemical characteristics and polarities that determine their solubility in a particular solvent (Taslimi *et al.,* 2020). The different solvent polarities play a significant role in determining the efficacy of the extraction process and the medicinal activity of the obtained extract (Barchan *et al.,* 2014). For the accurate quantification of antioxidant potential, the extraction of polyphenols from *M. odorata* pulp must be adequately optimised. Therefore, the present work focuses on the determination of nutritional composition and antidiabetic activity of *M. odorata* pulp extracted in different solvents. Further characterisation of polyphenols in the various extracts was conducted using ultra-high-performance liquid chromatography-electrospray ionisation orbitrap tandem mass spectrometry (UHPLC-ESI-Orbitrap MS/MS). This would provide beneficial information on the health benefits of *M. odorata*. Besides, findings from the present work are also expected to provide insights to researchers to explore the benefits of other underutilised tropical fruits.

Materials and methods

Plant materials

M. odorata fruits were purchased from a fresh fruit supplier in Serdang, Selangor, Malaysia. The authentication of the fruits was conducted by a botanist at the Institute of Bioscience, Universiti Putra Malaysia, Malaysia. The voucher no. is SK 3179/17. The peel was removed, and the pulp was sliced into thin pieces. The samples were lyophilised using freeze dryer (Labconco, MO, US), and ground to a fine powder using a grinder (Panasonic, MX-GM1011, Japan) at room temperature (24°C). The powder was stored in a sealed container, covered with aluminium foil to minimise exposure to light and oxygen. All samples were kept at -20°C until further analysis. Prior to extraction, sampling by the quartering technique was performed.

Proximate and dietary fibre analysis

Moisture content was determined by the vacuum oven method as outlined by Malaysian Standard (MS 1191:1991) (Lasano *et al.,* 2019b). Ash was determined by direct analysis (method 940.26) according to the Association of Official Analytical Chemists' methods (AOAC, 2000). Crude protein and fat contents were determined according to Tecator Manual and Soxtec Manual, respectively (AOAC, 2000). Total carbohydrate was determined by subtracting the total percent values of other measurements from 100. Energy was determined based on weight of macronutrients using the following factor: carbohydrate, protein, fat, and dietary fibre of 4, 4, 9, and 2 kcal/g, respectively. Proximate analyses were expressed as g per 100 g of fresh weight.

Total, insoluble, and soluble dietary fibres (DF) were determined using the AOAC enzymatic-gravimetric official method (985.29) (AOAC, 2000). The method uses heat stable α-amylase, amyloglucosidase, and protease treatments. The procedures were applied to remove protein and starch. In order to determine insoluble fibre, the samples were filtered and washed with acetone, 95% ethanol, and water. Then the samples were dried and weighed. Four volumes of 95% ethanol (preheated to 60°C) were added to the filtrate and to the water washings. Then, the precipitates were filtered and washed with 78% ethanol, and 95% ethanol and acetone. After that, the residues (soluble DF) were dried and weighed. The sum of total insoluble DF and soluble DF represents total DF.

Quantification of simple sugars

Simple sugars were determined according to a method described by Hernandez *et al.* (1998). A mixture of 10 g of dried samples and 80% methanol (50 mL) was refluxed in water bath (WiseBath WSB-18, South Korea) for 30 min at 95°C. The residue was then rinsed with 80% methanol (50 mL) after cooled down, and filtered. Then, the pooled extract was concentrated until approximately 20 mL solution remained using a vacuum rotary evaporator (Eyela, N-1000, Japan) at 50°C. Deionised water was mixed with sample until the solution became 25 mL. Before injecting into the system, the sample was filtered using 0.45 µm nylon membrane filter.

The chromatographic analysis was carried out using Waters 600 HPLC instrument with PhenoSphere 5 µm NH2 80A LC Column 250 × 4.6 mm (Merck, Kenilworth, New Jersey, USA). The system was equipped with Waters 600 Controllers (Waters Co. Ltd., Milford, MA, US) and refractive index (Waters 410 Differential Refractometer).The HPLC analysis was conducted using mobile phase mixture of acetonitrile:deionised water (80:20, v/v) in isocratic elution at a flow rate of 1.0 mL/min for 20 min, and injection volume of 10 µL. Sugar standards (fructose, glucose, and sucrose) were prepared by dilution with deionised water to different concentrations which ranged from 0.25 to 2.50% (w/v), and the calibration curve for each sugar was constructed.

Mineral content

The sample preparation for mineral analyses

was performed based on Zasoski and Burau (1977), using atomic absorption spectrophotometer (AAS) flame (SpectrAA 110, Varian, Melbourne, Australia). Briefly, 0.5 g of sample was added in the digestion tube. After that, HNO_3 was mixed with $HCLO_4$ (2:1, v/v). Then, the mixture (6 mL) was added to the sample. The digestion was done at 210°C. In order to calculate the concentration of the elements, calibration curve of each element was constructed. Analyses were performed in triplicate. The results were reported in mg per 100 g of sample on a dry weight basis (mg per 100 g DW).

Determination of total carotenoid content (TCC)

The TCC was determined according to the procedures outlined by Khoo *et al.* (2008). Briefly, 0.5 g of sample was mixed with 15 mL of hexane. The mixture was vortexed, and then centrifuged (Hettich EBA 20, Germany) at 3,000 rpm for 1 min. The extract was collected, and the extraction process was repeated using the sediment until it became colourless. The pooled extract was then evaporated to dryness using a vacuum rotary evaporator (Eyela, N-1000, Japan). Lastly, the dry extract was re-dissolved in 3 mL of hexane. The solution was read at 450 nm using a spectrophotometer (UV-1650 PC Spectrophotometer, Shimadzu, Japan). A calibration curve was constructed using β-carotene standard solution, and results were expressed as mg β-carotene equivalent per 100 g dry sample.

Determination of vitamin A

The vitamin A content was determined based on AOAC official method (method 960.45) using reverse-phase HPLC (AOAC, 2000). The filtered sample and standards were analysed on HPLC system equipped with Column C18, acetonitrile, methanol, and ethyl acetate at ratio 88:10:2, respectively, with injection volume 20 µL at flow rate 1.3 mL/min. Before that, the sample undergoes alkaline hydrolysis before extraction. Briefly, 20 g sample was added to a conical flask containing 80 mL of 95% ethanol and 20 mL of 20% potassium hydroxide (KOH). A few boiling chips was added before the mixture was refluxed for 30 min. After the process, the hydrolysate was extracted with 50 mL of hexane. The extraction step was repeated for three times, then it was washed with distilled water and anhydrous sodium sulphate. After that, the extract was evaporated to dryness in order to remove the solvent. After filtration using a 0.45 µm nylon membrane filter, the sample was injected into the HPLC system. β-carotene was used to develop a standard curve. The results were reported in mg per 100 g dry sample.

Determination of vitamin C

Vitamin C was determined using a titrimetric method according to AOAC official method 967.21 (AOAC, 2000). Briefly, 10 g of sample was mixed in a solution containing 40 mL of HOAc, in 500 mL of deionised water and 15 g of HPO_3. Then, the mixture was filtered in a 250 mL conical flask with a funnel and filter paper. Then, titration of the test samples and blank were performed with indophenol reagent until they turned to rose pink which lasted for less than 5 s. L-ascorbic acid was used as standard. Ascorbic acid content in the sample was estimated using Eq. 1:

$$\text{Mg ascorbic acid per 100 g sample} = X \times A \times V/Y \times 100/W \quad \text{(Eq. 1)}$$

where, X = mL of indophenol used to titrate the sample, A = mL of indophenol used to titrate the ascorbic acid standard (equivalent to mg of ascorbic acid contained), V = total volume (mL) of sample used, Y = total volume (mL) of sample used in titration to pink colour (10 mL), and W = weight of sample (g).

Determination of vitamin E

Vitamin E was determined according to procedures outlined in AOAC official method 971.30 (AOAC, 2000). Briefly, 2-10 g of samples were mixed with 0.25 g of ascorbic acid, 50% potassium hydroxide, and 50 mL of 95% ethanol in a flask. Then, the mixtures were refluxed at 40°C for 30 min. After being cooled, the solution was rinsed with distilled water (50 mL) in the separating funnel. Then, the solution was shaken vigorously after being mixed with petroleum ether (25 mL). Two-layer was formed, and the petroleum ether was collected by removing the lower layer into a beaker. The lower layer was added to the separating funnel again, and the extraction step was repeated by adding 25 mL of petroleum ether for another two times. The petroleum ether extracts were pooled and washed with water until the solution became neutral, and then it was filtered through anhydrous sodium sulphate. The petroleum ether extracts were then evaporated to dryness under nitrogen gas. After dilution with methanol, the solution was filtered using 0.45 µm membrane filter prior to HLPC injection at a flow rate of 1.0 mL/min. A reverse-phase HPLC-FLD system equipped with C_{18} and a fluorescence detector was used. The wave wavelength was set at 296 nm for excitation, and 330 nm for emission. The mobile phase used was methanol:deionised water (95:5). The gradient column pump mode was set at 1 - 5 min 95% A, 5.5 - 6 min 97% A, 6 - 25 min 95% A. Standard α-tocopherol at various concentrations were used to construct the calibration curve. Vitamin E was then

calculated using Eq. 2:

$$\text{Vitamin E (mg/kg)} = C \times 10/Ws \quad \text{(Eq. 2)}$$

where, C = concentration from the calibration curve (ppm), and Ws = sample weight (g).

α-glucosidase and α-amylase inhibition assay

Sample extraction

The sample extraction was carried out according to the method of Addai *et al.* (2013) with slight modifications, whereby the extraction hour was increased. Briefly, 1 g of dried fruit was mixed under magnetic stirring (Labtech LMS-1003, South Korea) with 10.0 mL of the solvent for 2 h (previously 1 h) in the dark at 24°C. Following centrifugation (Hettich EBA 20, Germany) for 15 min at 6,000 rpm, the extract was collected by filtration using a filter paper. The residue was washed with the same solvent (5 mL), followed by shaking at 6,000 rpm for 30 min. After the supernatant was pooled, the extracts was firstly dried using the rotary evaporator (Eyela, N-1000, Japan) at 40°C, and next by using freeze dryer (Labconco, MO, USA). For further analysis, the freeze-dried sample was re-dissolved at desired concentration using the same solvent extraction. 100% distilled water, acetone, methanol, and ethanol at 60% (v/v) concentrations were used as the extraction solvents. All the analyses were conducted in triplicate measurement.

α-amylase inhibition assay

The α-amylase inhibition assay was conducted based on the procedures reported by Kusano *et al.* (2010) and Chakrabarti *et al.* (2014) with slight modifications, whereby volume of iodine reagent used, enzyme concentration, and pre-incubation time were increased. The undigested starch due to enzyme inhibition was detected at 630 nm (blue, starch-iodine complex). The substrate (starch) was prepared by dissolving 200 mg soluble starch in 100 mL boiling deionised water (0.2%). Pre-incubation at 37°C for 10 min was done for the following chemicals: 20 µL of acarbose (100 µg/mL) or fruit extract (0.1 g/mL) at five concentrations (serially diluted into five concentrations), followed by 40 µL of substrate solution, and 20 µL of 0.6 U/mL α-amylase (20 mM phosphate buffer saline, pH 6.9). Then the mixtures was incubated for 15 min at 37°C. The reaction was stopped by adding 80 µL of iodine reagent (2.5 mM) and 80 µL of HCI (0.1 M). The measurement of absorbance was done using a microplate reader (Biotek, EL800, Winooski, US) at 630 nm. Acarbose was used as a positive control. All analyses were conducted in triplicate. α-amylase inhibition (%) was determined using Eq. 3:

$$\% \text{ inhibition} = \{(A_{sample} - A2)/ (A_{blank} - A_{control})\} \times 100 \quad \text{(Eq. 3)}$$

where, A_{sample} = the absorbance of the incubated mixture containing plant extract, starch, and amylase, A2 = the absorbance of incubating mixture of sample and starch, A_{blank} = the absorbance of incubated solution containing starch, and $A_{control}$ = the absorbance of the incubated mixture of starch and amylase. The IC_{50} value indicates the concentration of sample required to inhibit 50% enzyme activity.

α-glucosidase inhibition assay

The α-glucosidase inhibitory activity was measured based on the procedures reported by Al-Zuaidy *et al.* (2016). A 96-well plate, 10 µL of samples at five concentrations (the sample extracts at 0.1 g/mL was serially diluted into five concentrations) that was pre-incubated at 4°C for 30 min with 0.1 M phosphate buffer (pH 6.9) (70 µL), and 1 U/mL α-glucosidase enzyme (10 µL) were used. Then, the solution was mixed with 10 mM PNPG (*p*-nitrophenyl glucopyranoside) (10 µL). The absorbance was read at 405 nm after five min incubation at 37°C, using an ELISA microplate reader (Biotek, EL800, Winooski, USA). The positive controls were quercetin (60 µg/mL) and carbose (100 µg/mL). The percentage of enzyme inhibition was calculated using Eq. 4:

$$\% = [(\text{absorbance control} - \text{absorbance sample})/\text{absorbance control}] \times 100 \quad \text{(Eq. 4)}$$

The IC_{50} value represents the concentration of inhibitor required to achieve 50% enzyme inhibition.

Phytochemical profiling using UHPLC-ESI-Orbitrap-MS/MS

Sample preparation

Firstly, 1 mg/mL of sample extract was prepared and filtered using 0.2 µm nylon syringe filter. Next, for the standards, the concentrations were wet at 10 ppm by dissolving in methanol. The 16 targeted compounds included: (1) gallic acid, (2) protocatechuic acid, (3) vanillic acid, (4) catechin, (5) ethyl gallate, (6) epicatechin, (7) mangiferin, (8) p-coumaric acid, (9) ellagic acid, (10) myricetin, (11) apigenin, (12) kaempferol, (13) trans-ferulic acid, (14) trans-cinnamic acid, (15) quercetin, and (16) chlorogenic acid.

Identification of targeted polyphenols

The targeted polyphenols were identified based on Dorta *et al.* (2014). The sample extract and mixed standards were analysed using UHPLC-Ultimate 3000 system (Dionex, Sunnyvale, California, USA), with an autosampler and PDA detector. The U-HPLC column (100 × 2.1 mm, 1.9 um, Hypersil Gold) (ThermoFisher Scientific, Waltham, Massachusetts, USA) was used for the separation. Solvent (A) consisted of 0.1% formic acid in water (v/v), while solvent (B) was 0.1% formic acid in acetonitrile at a flow rate of 0.4 mL/min.

Mass spectrometry was performed using a Thermo Electron Q Exactive Focus-Orbitrap mass spectrometer equipped with a heated electrospray ion source (ThermoFisher Scientific, Waltham, Massachusetts, USA). The MS operated under Xcalibur 4.0 version software. The spectra were recorded in the range of *m/z* 50 - 7590 with a resolution of 70,000, and the sheath and auxiliary gas had a flow rate of 40 and 50 arbitrary units, respectively.

To further identify the compound, firstly, a full scan MS mode was applied. Then, data-dependent scan was conducted in order to get the MS/MS spectra, and to select the most intense ion or specified ions (accurate mass of authentic standard). The confirmation of the data-dependent scan for MS/MS was done using the isolation window 2.0 amu and resolution of 17,500. The instrument was calibrated every 7 d according to the manufacturer's calibration standards. The compounds were identified by comparison with the reference compounds and with literature data from retention times, PDA, spectra MS, and MS/MS (fragment ions) analysis. In order to compare the MS/MS spectra, we used the literatures and several online databases (ChemSpider, Raleigh, North Carolina, USA).

Statistical analysis

The statistical analysis was performed using Minitab version 16.0 (Minitab Pty Ltd, Sydney, Australia). Analysis on proximate composition, dietary fibres, simple sugars, and antioxidant vitamins were carried out in duplicates, while antidiabetic activities were conducted in triplicates. All data were presented as mean ± standard deviation. Analysis of variance (ANOVA) followed by Tukey's HSD test were conducted to identify differences of mean values. Significance level was set at $p < 0.05$.

Results

Nutritional composition of M. odorata pulp

The proximate composition, sugars, minerals, and antioxidant vitamins in *M. odorata* pulp are presented in Table 1. Results revealed that the pulp contained high moisture (84.20 ± 0.10%), and may provide a great source of carbohydrate (14.50 ± 0.09%) and total dietary fibre (15.71 ± 1.27%), particularly the soluble fibre (11.06 ± 0.21%). Additionally, there was only 0.90 ± 0.01% crude protein, and no fat was detected in the pulp. Analysis on the individual sugar content found that sucrose was the main simple sugar in the pulp (13.41 ± 0.92%), followed by fructose (3.89 ± 0.53%) and glucose (1.39 ± 0.33%). In our previous work, we described the antioxidant activity of *M. odorata* fruit (Lasano *et al.,* 2019a). In the present work, we focused on the determination of antioxidant vitamins. Based on Table 1, the total carotenoid content (TCC) in *M. odorata* pulp was 450.00 ± 40.00 mg/100 g dry weight. High performance liquid chromatography (HPLC) analysis found that β-carotene (78.58 ± 0.09 mg/100 g dry weight) was the highest antioxidant vitamin in the pulp, which also reflects the pigment responsible for the fruit colour. The major mineral in *M. odorata* pulp was potassium (1012.52 ± 5.91 mg/100 g dry weight), with calcium (106.38 ± 2.35 mg/100 g dry weight), magnesium (103.20 ± 0.44 mg/100 g dry weight), and phosphorus (90.27 ± 0.45 mg/100 g dry weight) were also found in significant amount. The nutritional composition data of *M. odorata* pulp suggested that the fruit is rich in dietary fibre, antioxidant vitamins, and minerals which could be a source of essential nutrients, and eventually improve human diet.

Effects of different extraction solvents on antidiabetic activity of M. odorata pulp

The inhibition of carbohydrate-hydrolysing enzymes such as α-glucosidase and α-amylase could slow the absorption of glucose in diabetic people, thus resulting in lowered blood glucose level (Sarmadi *et al.,* 2012). We, therefore, conducted α-glucosidase and α-amylase inhibitory assays in order to determine the antidiabetic potential of *M. odorata* pulp extracted in acetone, ethanol, and methanol at 60% (v/v). The IC_{50} values (mg/mL) are displayed in Table 2. The plant extract with higher inhibitory activity is represented by lower IC_{50} value. The sample extracted in 60% ethanol showed the lowest IC_{50} value as compared to other extracts. On the other hand, the IC_{50} values for α-glucosidase inhibitory activity were not detected irrespective of

Table 1. The proximate composition, total dietary fibres, sugars, antioxidant, vitamins, and minerals content in *M. odorata* pulp.

Composition	Value
Proximate analysis (%)	
Moisture	84.20 ± 0.10
Crude protein	0.90 ± 0.01
Crude fat	nd
Carbohydrate	14.50 ± 0.09
Ash	0.43 ± 0.05
Energy (kcal)	61.50 ± 0.71
Fibre (%)	
Total dietary fibre	15.71 ± 1.27
Soluble fibre	11.06 ± 0.21
Insoluble fibre	4.65 ± 1.05
Sugar (%)	
Fructose	3.89 ± 0.53
Glucose	1.39 ± 0.33
Sucrose	13.41 ± 0.92
Antioxidant vitamin (mg/100 g dry weight)	
Total carotenoid content (TCC)	450.00 ± 40.00
ß-carotene	78.58 ± 0.09
Ascorbic acid	3.90 ± 0.00
α-tocopherol	1.07 ± 0.00
Major mineral (mg/100 g dry weight)	
Potassium	1012.52 ± 5.91

All values are presented in mean ± standard deviation (n = 2), except for sugar (n = 3). nd = not detected.

Table 2. α-amylase and α-glucosidase inhibitory activities of *M. odorata* pulp extracted in different extraction solvents.

Extract	IC_{50} value (mg/mL)	
	α-amylase	α-glucosidase
60% acetone	nd	nd
60% ethanol	20.86 ± 0.32	nd
60% methanol	52.18 ± 1.29	nd
Water	51.22 ± 9.02	nd
Acarbose	0.067 ± 1.84	0.081 ± 4.17
Quercetin	-	0.046 ± 0.23

All values are presented in mean ± standard deviation (n = 3). The solvents used for extraction was 60% (v/v), except for water. nd = not detected.

the solvents used. The data suggested that 60% ethanolic extract may contain active compounds; hence, the phytochemical profiling was conducted using the ethanolic extract of *M. odorata* pulp.

Identification and confirmation of targeted polyphenols in the ethanolic extract of M. odorata pulp using UHPLC-ESI-Orbitrap-MS/MS

The targeted polyphenols were identified based on the predominant polyphenols in M. indica (Abdullah *et al.,* 2014). The retention times, $[M-H]^-$ ion, and MS/MS (MS^2) spectra (Figure 1) were compared with 16 standards for the confirmation of the compounds in the *M. odorata* pulp extract. Table 3 presents the tentative assignment of the compounds, the retention time (RT), MS and MS^2 fragments, observed *m/z*, as well as their relative abundance. Peaks 1, 2, 3, and 5 were identified as phenolic acid specifically hydroxybenzoic acid. Among the peaks, peak 1 was identified as gallic acid. The peak gave $[M-H]^-$ ion at *m/z* 169.01, and main fragment ion at *m/z* 125 was created after the loss of a $-CO_2$ group (Chernonosov *et al.,* 2017).

Peak 2 was identified as protocatechuic acid or 3,4-dihydroxybenzoic acid. Peak 2 has deprotonated ions $[M-H]^-$ at *m/z* 153.01, and showed the main fragment ions at *m/z* 109 and 108. The fragment ions at *m/z* 109 was identified as resulted from the common loss of a $-CO_2$ thus giving [M-H-44]- as a characteristic ion (Vallverdú-Queralt *et al.,* 2011). In addition, vanillic acid (peak 3) was also identified. Peak 3 gave $[M-H]^-$ at *m/z* 167.03 and showed fragment ions at *m/z* 152.0, 108.0, 91.0,

Table 3. Chromatogram and spectral properties of compounds detected in 60% ethanolic extract of *M. odorata* pulp by LC-ESI Orbitrap-MS/MS, and their relative abundance.

Peak	t_R (min)	$[M-H]^-$ (*m/z*)	MW	Fragment ions MS^2, m/z (abundance)	Compound	Relative abundance
1	1.42	169.01	170.12	125.0 (100), 124.0 (24), 67.0 (28)	Gallic acid	19.34
2	2.57	153.01	154.12	109.0 (100), 108.0 (80)	Protocatechuic acid	19.89
3	6.65	167.03	168.04	152.0 (88), 108.0 (100), 91.0 926), 65.0 (58)	Vanillic acid	21.38
4	7.22	289.07	290.20	289.1 (30), 245.0 (18), 203.0 (14), 151.0 (14), 137.0 (28), 123.0 (70), 109.0 (100), 97.0 (24), 93.0 (20), 83.0, (20), 57.0 (30)	Catechin	47.24
5	8.26	197.04	198.17	197.0 (60), 169.0 (74), 140.0 (24), 125.0 (100)	Ethyl gallate	44.80
6	8.28	289.07	290.20	289.0 (30), 245.0 (34), 203.0 (30), 151.0 (30), 137.0 (30), 123.0 (74), 109.0 (100), 97.0 (30), 81.0 (24), 69.0 (24), 57.0 (36)	Epicatechin	47.68
7	8.38	421.07	422.23	421.7 (38), 331.0 (70), 301.0 (100), 272.0 (60), 271.0 (66), 259.0 (50), 258.0 (58)	Mangiferin	61.31

Peak = compound number; t_R = retention time (min); $[M-H]^-$ = deprotonated ion; MW = molecular weight; Fragment ions = the product ions produced from MS/MS full scan.

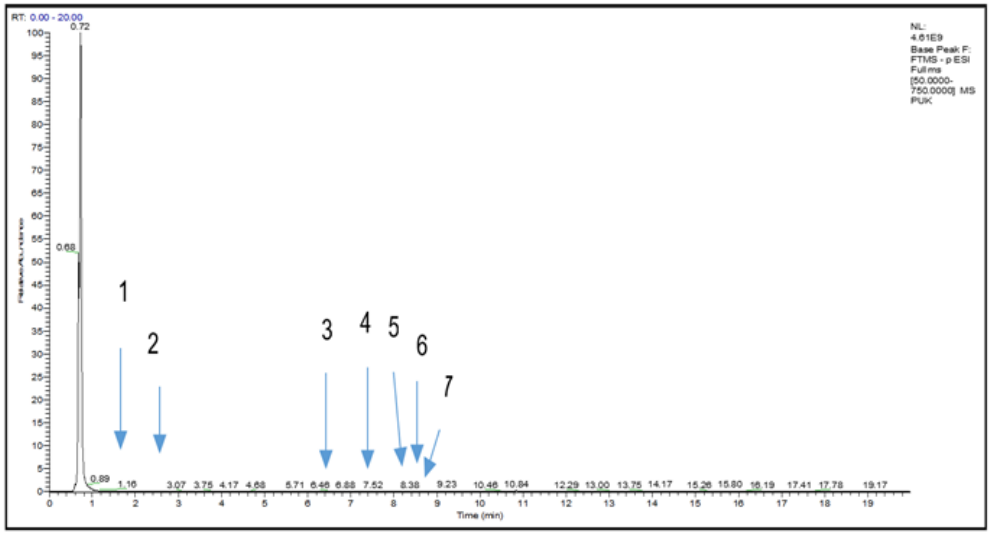

Figure 1. Total ion chromatogram of 60% ethanolic extract of *M. odorata* pulp by LC-ESI-Orbitrap-MS/MS.

and 65.0. MS^2 of the precursor ion yielded product ion at m/z 152, 108 and 91, which is attributed to the loss of a methyl group [M-H-15]- and further loss of a $-CO_2$ [M-H-44]- and –OH $[M-H-17]^-$, respectively (Horai et al., 2010).

Peak 5 that showed the $[M-H]^-$ ion at *m/z* 197.04 was identified as gallates or also known as ethyl gallate. The fragment ions produced from ethyl gallate was shown at *m/z* 197.01, 169.0, 140.0, and 125.0. The major fragments of ethyl gallate at *m/z* 169 were produced after the loss of $-CH_2{=}CH_2$ $[M-H-28]^-$ and the fragment ion indicated the presence of gallic acid. Moreover, the fragment ion at *m/z* 125 was generated after the missing of $-CH_2{=}CH_2$ and $-CO_2$ $[M-H-28-44]^-$ (Dorta *et al.,* 2014). Besides, flavanol was also found in the *M. odorata* pulp extracts.

Catechin and epi-catechin were identical to peaks 4 and 6, respectively. Catechin $[M-H]^-$ at *m/z* 289.07 yielded fragment ions at 289.1, 245.0, 203.0, 151.0, 137.0, 123.0, 109.0, 97.0, 93.0, 83.0, and 57.0. The isomer epi-catechin gave the same fragment ions, as the stereoisomers could not be distinguished by mass spectrometry. Fragmented ions were slightly similar to previous studies (Lopes-Lutz *et al.,* 2010), where the fragment ions at *m/z* 245, 205, and 179; and 271, 245, 205, and 139, respectively. These showed that the fragmentation process was not completed since the precursor ion the present work was still in high abundance. Peak 7 was identified as

mangiferin ([M-H]$^-$ at *m/z* 421.07. It showed fragment ions at *m/z* 421.7, 331.0, 301.0, 271.0, 259.0, and 258.0 as the main peak.

Discussion

Although the pharmacological properties of *Mangifera* species have been well documented (Torres-León *et al.,* 2016), limited data is available regarding the chemical composition and *in vitro* antidiabetic activities of *M. odorata*. As aforementioned, *M. odorata* displayed potent antioxidant activity, and the by-products from the fruit could be processed into functional food ingredients, owing to ample source of essential nutrients and phytochemicals (Lasano *et al.,* 2019b). In the present work, the proximate analysis, mineral, vitamin, and sugar contents, along with the *in vitro* antidiabetic activities in different extraction solvents, as well as the phytochemical profiling of the extract of *M. odorata* pulp were determined. Analysis on the nutritional properties of *M. odorata* pulp revealed a high moisture content (84.20 ± 0.10%) indicating that drying process is needed in order to ensure the microbial safety and quality of the fruit. Besides, the findings from the present study were almost comparable with the moisture content in the pulp of passion fruit (88.1%), pineapple (86.9%), and papaya (87.8%) (Morais *et al.,* 2017). In contrast to earlier findings, however, no fat was detected in the fruit pulp of *M. odorata* which may result in lower calorie content (Mirfat *et al.,* 2015). On the other hand, mango varieties of other species such as *M. indica*, namely Haden and Tommy, were reported to contain an appreciable amount of fat (0.29 and 0.27%, respectively) (Bello-Pérez *et al.,* 2007). In addition, the protein and carbohydrate content in the *M. odorata* pulp is reported to be lower than avocado, pineapple, banana, papaya, and passion fruit (Morais *et al.,* 2017).

The findings also highlighted that *M. odorata* pulp could be a significant source of dietary fibre, particularly the non-digestible carbohydrates. The levels of dietary fibre, especially soluble fibre observed in the present work are far higher than those reported in a similar study by Tee *et al.* (1997). A combination of several types of dietary fibre comprising of insoluble and soluble fibre is an essential component of a healthy and balanced diet. Generally, insoluble fibre increases faecal bulk, and is important for caloric content of food, while soluble fibre decreases blood cholesterol and may improve metabolic profiles of a diabetic patient (Chen *et al.,* 2016). Mostly, in mature mango, glucose, fructose, and sucrose form the major proportion of carbohydrate (Desnoues *et al.,* 2014). In the present work, sucrose was found to be the major type of sugar in *M. odorata* pulp, followed by fructose and glucose.

Besides, the present work also found a considerably high amount of total carotenoid content (TCC) in the *M. odorata* pulp as compared to the value of 3.95 mg/100 g that was reported by Khoo *et al.* (2008). In addition, *M. odorata* has a higher vitamin A content as compared to papaya (32.17 µ g/100 g), mango (35.67 µg/100 g), and watermelon (11.34 µg/100 g) (Tee *et al.,* 1997). The major mineral element identified in *M. odorata* fruit is potassium, where the potassium level was reported to be higher than those found in Mexican apples, avocado, grapes, lime, lemon, mango, plum, orange, papaya, and pineapple (Sanchez-Castillo *et al.,* 1998). Hence, *M. odorata* pulp can be a significant source of potassium. This finding is conflicting with a previous study which found lower potassium (635 mg/100 g), calcium (95 mg/100 g), and magnesium (75 mg/100 g) in *M. odorata* pulp, but a higher concentration of zinc and iron (2.2 and 3.17 mg/100 g, respectively) (Tee *et al.,* 1997). On the whole, the nutritional properties findings suggest that *M. odorata* pulp could supply some essential nutrients that are beneficial for the human diet.

Furthermore, the ability of plant extracts as antidiabetic agents, particularly to reduce post-prandial blood glucose has been indicated in both *in vivo* studies and human clinical trials (Modak *et al.,* 2007). This could be attributed to the presence of α-amylase and α-glucosidase inhibitors in the plant extracts, which increases the digestion time and eventually delays the glucose absorption from the intestinal cells (Sarmadi *et al.,* 2012) Therefore, in the present work, the α-glucosidase and α-amylase inhibitory activity of *M. odorata* pulp extracted in different solvents, namely methanol, acetone, ethanol, and pure deionised water were investigated. The findings demonstrated that 60% of ethanolic extract showed the highest α-amylase inhibition. Similarly, a previous study has demonstrated that *M. indica* ethanolic extract inhibited α-glucosidase and α-amylase activities and improved the metabolic profiles of streptozotocin-induced diabetic rats by lowering fasting blood glucose, glycated haemoglobin levels, and increased plasma insulin level (Gondi *et al.,* 2015). Furthermore, the present findings showed no inhibition of α-glucosidase in all of the pulp extracts indicating the absence of α-glucosidase inhibitors in the *M. odorata* pulp. Further, the antidiabetic activity of *M. odorata*

extract was compared with the standard (acarbose), and it was noted that all of the extracts showed lower IC_{50} values as compared to the standard. Nevertheless, the potential α-amylase inhibitory activity of *M. odorata* pulp may decrease the amount of sugar absorbed into the bloodstream and help to prevent hyperglycaemia, probably with reduced side effects as compared to synthetic drugs. Thus, it is important to further identify the bioactive compounds responsible for the observed antidiabetic activity, particularly in the ethanolic extract of *M. odorata* pulp.

A recent study has reported the presence of compounds such as mangiferin, myricetin, ellagic acid, epicatechin, catechin, kaempferol, apigenin, and gallic acid in the by-products of *M. odorata* which are the peel and seed kernel (Lasano *et al.,* 2019b). Additionally, an investigation on the functional content of *M. indica* revealed the presence of rutin, catechin, gallic acid, and ellagic acid (da Silva Sauthier *et al.,* 2019). These compounds have exhibited a hypoglycaemic effect through the inhibition of α-amylase and α-glucosidase activities (Gondi *et al.,* 2015). The present work has identified mangiferin as the most abundant compound in the pulp extract. Meanwhile, mangiferin has also been previously reported as the main polyphenol in mango bark, kernel, leaves, and peel (Barreto *et al.,* 2008). Mangiferin content in the crude extract was also described as a potent antidiabetic agent, even at a low concentration of 50 μg/mL (Kulkarni and Rathod, 2018). Further, the present work also found vanillic acid and protocatechuic acid, which were not identified in any of the earlier studies (da Silva Sauthier *et al.,* 2019). However, protocatechuic acid was detected in the acetone extract of the mango peel (Gondi *et al.,* 2015). Thus, it is hypothesised that the different polarities of ethanol and acetone may account for the variances in the extracted compounds. Moreover, flavanols (catechin and epicatechin), gallic acid, and ethyl gallate were found in moderate amounts in the *M. odorata* pulp.

Conclusion

Ultimately, the results of the present study will unravel and shed light on the understanding of the potential active compounds in *M. odorata* fruit pulp in lowering post-prandial blood glucose. Thus, it is postulated that the mangiferin-rich ethanolic extract of the pulp might contribute to the α-amylase inhibition activity. Further work using more appropriate or advanced techniques can be conducted to determine and quantify other unidentified compounds. Additionally, for compounds which could not be distinguished by using mass spectrophotometry, isolation of the compounds would be beneficial. It is also crucial to elucidate the mode of action of the plant extracts in inhibiting the α-amylase activity. To this end, *in vivo* testing on antioxidant and antidiabetic properties must be accomplished to confirm and support the current findings. Moreover, bioavailability and interactions study of the detected bioactive compounds could be further explored to determine their efficacy in alleviating oxidative stress-related diseases.

Acknowledgement

The present work was financially supported by Universiti Putra Malaysia (UPM) Putra Grant scheme under the Putra Muda Initiative (GP-IPM 2016/9474600). The authors would like to thank the Laboratory of Functional Food, Faculty of Food Science and Technology for technical support received in the completion of the present work.

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