Correlation of the GC-MS-based metabolite profile of *Momordica charantia* fruit and its antioxidant activity

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Abstract

Momordica charantia or bitter melon (Cucurbitaceae) is a widely consumed edible fruit with strong antioxidant properties. Due to these properties, it has been commercialised by the natural product industries as a coadjutant in the treatment of various ailments attributable to the deleterious effects of oxidants. The present work aimed to evaluate the antioxidant activity of *M. charantia* fruit extracts made with different compositions of ethanol:water, and to identify the metabolites that are responsible for this activity. To this end, the fruit samples were extracted using six different concentrations of ethanol in water (0, 20, 40, 60, 80, and 100%). Gas chromatography-mass spectrometry (GC-MS) and multivariate data analysis (MVDA) were used to identify significant antioxidants. The 80% ethanol:water extract showed the most significant ($p < 0.05$) antioxidant activity when tested with the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) antioxidant assays. The multivariate data analysis revealed that the metabolites related to this antioxidant activity were gentiobiose, glucose, galactonic acid, palmitic acid, galactose, mannose, and fructose.

Introduction

The prevalence of diabetes mellitus (DM) has been escalating. It was estimated that in 2030, Malaysia would have a total number of 2.48 million cases. As a result, the healthcare expenditure on DM accounted for 16% of the total healthcare expenditure in Malaysia; equivalent to USD 1.01 million annually, as DM becomes one of the major problems faced by the country (Ashari *et al.,* 2016).

It is well known that the oxidative stress resulting from hyperglycemia is closely related to the development and progression of DM and its related complications. The aetiology of DM through oxidative stress is assumed to be due to the increased production of free radicals or impaired antioxidant defences (Yaribeygi *et al.,* 2019). Mechanisms of the diabetic complications where increased oxidative stress is involved in are through the activation of transcription factors; advanced glycated end products (AGEs), a mitogen-activated protein kinase (Yang *et al.,* 2019; Dharshini *et al.,* 2020; Kim *et al.,* 2020).

Another mechanism that promotes the production of free radical is through the interaction of glucose with proteins which further increases the production of amadori product, followed by (AGEs). These AGEs, via their receptors (RAGEs), inactivate enzymes and alter their structures and functions.

They promote free radical formation as well as quench and block antiproliferative effects of nitric oxide. When intracellular oxidative stress is increased, AGEs activate the transcription factor viz. nuclear factor-beta cell (NF-β). This eventually activates the up-regulation of various NF-β-controlled target genes. NF-β enhances the production of nitric oxide which is believed to be a mediator of islet β-cell damage (Ansari and Dash, 2013).

The available synthetic drugs that are prescribed for diabetes have led to many side effects such as diarrhoea, nausea, vomiting, bloating, and flatulence among sensitive patients or after prolonged use. Besides, long-term use of synthetic drugs will eventually lead to complications involving major organs, especially kidney and liver (Alhadramy, 2016). Therefore, drug discovery leading to minimal side effects and less toxicity were recently implemented. One of the strategies is the use of natural medicinal herbs. Although herbs are considerably safer than commercial synthetic drugs and at the same time can treat diseases, scientific proof on the use of herbs for medicinal purpose is still lacking. Among the medicinal herbs, *M. charantia* fruit (bitter melon) has been reported to have antidiabetic and antioxidant activities (Gao *et al.,* 2018).

The analytical platform used in the present work was gas chromatography-mass spectroscopy (GC-MS), a technique used for metabolomics studies. It provides the high resolution needed to separate the components of a complex biological mixture, and is highly sensitive and cost effective. Due to these advantages, it has been increasingly used in the metabolite profiling of plants (Stettin *et al.,* 2020).

In the present work, the antioxidant activities of *M. charantia* fruit extracts prepared with different compositions of ethanol:water as a solvent was evaluated, and the metabolites related to these activities were then identified using GCMS-based metabolomics.

Materials and methods

Chemicals

Ethanol, hydrochloric acid, acetic acid, and acetone were purchased from R&M marketing (Essex, UK). Ascorbic acid, 1,1-diphenyl-2 picrylhydrazyl (DPPH, 95%), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ, 99%), iron (III) chloride hexahydrate, sodium acetate, pyridine, N-methyl-N-(trimethylsilyl) trifluoroacetamide purum (97.0%), and methoxyamine hydrochloride (98%) were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A).

Plant collection and extraction

Raw fruits (12-week old; 50 kg) of *M. charantia* were randomly collected from a farm located in Perak, Malaysia. A sample was deposited in the Herbarium of Kulliyyah of Pharmacy, International Islamic University of Malaysia on 8 June 2014 for species verification, with the voucher number PIIUM 0215. The seeds were removed from the fruits, and the fruits were washed, frozen in liquid nitrogen, ground to a fine powder, and frozen at -80°C.

Fruit samples were extracted with six different mixture ratios of ethanol:water (0, 20, 40, 60, 80, and 100%) in six replicates for each mixture ratio. For this, 5 g of the powdered freeze-dried fruits were placed in a conical flask with 150 mL of each ethanol:water mixture ratio, and sonicated for 30 min. After sonication, the extracts were paper-filtered (No. 1 Whatman International, Maidstone, UK), and the solvent was evaporated by a vacuum rotary evaporator (Buchi, Flawil, Switzerland) at 40 ± 1°C. The extracts were then freeze-dried and stored at -80°C until further analysis. The yield of extraction was calculated using Eq. 1:

$$\text{Yield of extraction (\%, w/w)} = \text{Wta} / \text{Wtb} \times 100\% \quad \text{(Eq. 1)}$$

where, Wta = weight of the dried crude extract, and Wtb = weight of the plant raw material.

DPPH radical scavenging activity

The DPPH radical scavenging activity of the six different *M. charantia* fruit ethanolic extracts was measured using the method adapted from Karthivashan *et al.* (2013). Briefly, 20 μL of the six different *M. charantia* fruit ethanolic extracts and ascorbic acid (standard) were separately added to 80 μL of DPPH solution, and the mixture was kept for 10 min in the dark at room temperature. The absorbance of the solution was then measured at 540 nm using a microplate reader (Tecan, Männedorf, Switzerland). A blank solution was prepared by mixing 20 μL of water with 80 μL of DPPH solution. The DPPH radical scavenging activity was calculated using Eq. 2:

$$\text{DPPH radical scavenging activity (\%)} = [(\text{ODcontrol} - \text{ODsample}) / \text{ODcontrol}] \times 100\% \quad \text{(Eq. 2)}$$

where, OD = optical density.

The IC$_{50}$ values of DPPH radical scavenging activity were calculated based on a calibration curve obtained by plotting the radical scavenging activity (%) *versus* various dilutions of sample/standard assay. The IC$_{50}$ was determined as the sample concentration that produced half-maximal inhibitory concentration. The experiment was performed using six replicates and their average, and the standard error of the mean (SEM) was used to calculate the final value.

FRAP assay

The total antioxidant capacity of the six different *M. charantia* fruit ethanolic extracts was determined using the FRAP method adapted from Szydłowska-Czerniak *et al.* (2011). Briefly, 2.5 mL FRAP reagent (10 mM TPTZ solution in 40 mM HCl), 2.5 mL of 20 mM FeCl$_3$, and 25 mL of 0.1 M acetate buffer pH 3.6 was prepared and incubated for 10 min at 37°C. Then 20 μL of each ethanolic extract, ascorbic acid (standard), and 40 μL of FRAP reagent were added to 140 μL of distilled water in a 96-well plate, thus producing a blue-colored solution. The solutions were kept at room temperature for 20 min in the dark, and then their absorbance was measured at 593 nm with a microplate reader (Tecan, Männedorf, Switzerland) using a reagent blank composed of 40 μL of FRAP reagents in 200 μL of distilled water. A calibration curve was prepared with a serial dilution of the ascorbic acid (standard). The total antioxidant capacity was calculated by interpolation of the measured absorbance in the calibration curve, and expressed as ascorbic acid equivalents (AAE). The results were corrected for dilution, and expressed as AAE μg of ascorbic acid/g.

Derivatisation procedure

All dried *M. charantia* fruit ethanolic extracts were derivatised with MSTFA (N-methyl-N-trimethylsilyl trifluoroacetamide) for GC-MS analysis based on Murugesu *et al.* (2018). The purpose of derivatisation is to produce alkyl-silylated derivatives from the polar and non-volatile compounds in the tested samples. For this, 25 mg of the dried samples were dissolved in 50 μL of pyridine, and placed in the incubator shaker at 30°C for 10 min. Next, 100 μL of methoxyamine (20 mg/mL pyridine) was added, vortexed for 10 min, and kept at 60°C for 2 h. A further 300 μL of MSTFA was added and left for 30 min at 60°C. Finally, the samples were filtered using a microsyringe filter, and transferred to a microvial for GC-MS analysis.

GC-MS analysis

GC-MS analysis was conducted according to Murugesu *et al.* (2018) with some modifications. Samples were analysed on an Agilent 6890 gas chromatograph connected to an Agilent 5973 quadrupole and mass selective detector. A volume of 1 μL was injected onto a DB-5MS, 5% phenyl methyl siloxane column with an inner diameter (ID) of 250 μm, and a film thickness of 0.25 μm. An auto-sampler (Agilent, Santa Clara, United States) was used with a splitless injection mode. Samples were eluted with a gradient starting at 170°C for 5 min, and then increased to 315°C at a rate of 10°C/min with a total run time of 20 min. The injector and ion source temperatures were set to 330 and 250°C, respectively. Helium gas was used as the carrier gas at a flow rate of 1.0 mL/min. Mass spectra were acquired using a full scan and a monitoring mode in a range of 50 to 550 m/z after a solvent delay of 6 min. A total of six replicates of each individual sample were applied. The obtained data were processed and analysed with the Agilent Chemstation G1701DA software. The National Institute of Standards Technology library (NIST, Gaithersburg, MD, USA) library was used as a database.

Multivariate data analysis

Raw chromatograms were converted into the computable document format (CDF). The preprocessing of all raw data into matrix data included systematic noise filtration, data binning, automatic peak detection, and chromatographic alignment. It is essential, and in the case of GC-MS, that the data does not require internal standards (ISs). Baseline correction of the total ion chromatogram (TIC), integration, and peak picking were done using the ACD/Spec Manager v.12.00 (Advanced Chemistry Development, Inc., ACD/Labs Toronto, Canada). The identification of metabolites was performed by matching their respective spectra with the NIST library. The XCMS package in R version 2.15.1 was applied to align the GC-MS chromatograms using the following parameters: xcms set (fwhm = 30, step = 0.1, method = bin) and group (bw = 10). The data on antioxidant activity was added to the converted Microsoft Excel format.

Quantification of tentative compounds by GC-MS

The quantification of tentative bioactive

compounds (gentiobiose, galactose, palmitic acid, mannose, glucose, galactonic acid, and fructose) using GC-MS analysis in the present work was conducted following the methods described by Murugesu *et al.* (2018) together with Omar and Salimon (2013) with some modifications. The bioactive compounds analysed were selected based on the result of MVDA on GCMS based spectra, as previously mentioned. The stock solution of these tentative bioactive compounds was prepared by dissolving 1 mg of each compound in 50 µL pyridine, and derivatised following the method previously described. A serially diluted solution of the sample was prepared by adding pyridine into the derivatised solution with a dilution factor of 2. Each serial dilution was prepared in triplicate. A calibration curve was constructed for each of the standards by plotting the peak area (*y*-axis) against the concentration of the standard (*x*-axis) in order to produce a linear regression equation. The limit of detection (LOD) and limit of quantification (LOQ) of each compound were determined as a signal-to-noise ratio of 3:1 and 10:1, respectively.

External standards were spiked into the *M. charantia* fruit ethanolic extract with the amount (W1) approximating to that of bioactive compounds in the extract prior to derivatisation as previously described. After derivatisation, 1 µL of the spiked sample was injected to GC-MS. The estimated amount (W2) of the external standard was calculated by substitution of its peak area through its linear regression equation constructed in the previous section. Recovery of the external standards (R) was calculated using Eq. 3:

$$R = (W2/W1) \qquad\qquad (Eq. 3)$$

Similarly, the estimated amount of the bioactive compounds (Wc) in the extract was calculated by substituting its peak area to its linear regression equation. The concentration of each compound (C) was calculated by considering the weight of the sample (Ws) using Eq. 4:

$$C = (Wc/Ws) \times R \qquad\qquad (Eq. 4)$$

Synergistic effect of suspected bioactive compounds

Tentative bioactive compounds were spiked individually into the dried 80% ethanolic extract with the amount 2-, 4-, and 8-time higher than the normal amount of these compounds. The DPPH radical scavenging activity and FRAP of the spiked samples were tested accordingly.

Statistical analysis

The GCMS/antioxidant activity data were imported into the SIMCA P+ 14.0 software (Umetrics AB, Umeå, Sweden) for MVDA. Orthogonal Partial Least Square (OPLS) was used to obtain the best discrimination of the sample based on the antioxidant activity and GC-MS profile. It was UV-scaled (unit variance) and centered to allow for OPLS modeling. The significant difference was determined by a one-way ANOVA test with a Tukey comparison at a confidence interval of 95% using Minitab 14 (Minitab Inc., State College, Pa., USA).

Results

Yield of extraction

Table 1 shows the yield of *M. charantia* fruit extracted with different concentrations of ethanol:water (0, 20, 40, 60, 80, and 100%). There were significant differences in the yields which ranged from 23.36 to 62.03%. The highest yield was obtained with 80% ethanol:water, while 40% yielded the lowest. There was no general trend observed. Thus, 80% ethanol:water was selected as the most suitable extraction solvent based on the yield (in mass) of the obtained extract.

Radical scavenging activity by DPPH assay

The radical scavenging activity by DDPH assay was found to be concentration-dependent. Table 1 shows the DPPH radical scavenging capacity of the extracts. The values were expressed as the IC_{50}. The extracts prepared with 80% ethanol:water exhibited the strongest inhibition of 0.37 mg/mL ($p < 0.05$) as compared to 0.02 mg/mL of ascorbic acid, while the lowest activity was found in extracts obtained with 20% ethanol:water and 100% of ethanol. This agrees with Qader *et al.* (2011) who observed that an aqueous extract of *M. charantia* fruit exhibited the weakest inhibition of DPPH free radical scavenging. The difference in IC_{50} values among the different concentrations of *M. charantia* extracts has been attributed to the differences in the metabolic profile obtained with solvents of different polarities (Javadi *et al.,* 2014).

Ferric reducing activity by FRAP assay

Table 1 shows that the 80% ethanol:water yielded the highest antioxidant capacity (113.85 AAE µg of ascorbic acid/g). This value is almost equal to the AAE value of ascorbic acid (114.58 AAE µg of ascorbic acid/g). This was followed by the extracts made with ethanol:water 100, 60, 40, 20, and 0% with 112.31, 86.11, 85.51, 65.32, and 54.27

Table 1. Comparison of extraction yield, FRAP value, and DPPH radical scavenging activity of *M. charantia* fruit.

Extract (% of ethanol in water, v/v) and positive control	FRAP (AAE µg of ascorbic acid/g)	DPPH IC$_{50}$ (mg/mL)	Extraction yield (%)
0	54.27 ± 0.60[d]	1.10 ± 0.08[a]	40.36 ± 3.23[b]
20	65.32 ± 0.23[c]	1.10 ± 0.04[a]	38.51 ± 1.10[b]
40	85.51 ± 1.04[b]	1.09 ± 0.08[a]	23.30 ± 1.26[d]
60	86.11 ± 2.16[b]	1.05 ± 0.10[ab]	35.32 ± 2.30[bc]
80	113.8 5± 0.94[a]	0.37 ± 0.07[d]	62.03 ± 1.27[a]
100	112.31 ± 1.34[a]	0.53 ± 0.06[cd]	24.71 ± 3.14[cd]
Ascorbic acid	114.58 ± 1.73[a]	0.02 ± 0.01[e]	ND

Values are mean ± standard error mean (SEM) of six replicates (*n* = 6). Means in each column with different lowercase superscripts are significantly (*p* < 0.05) different. ND: not determined.

AAE µg of ascorbic acid/g, respectively. As compared to free radical scavenging activity, the ferric reducing capability of the *M. charantia* extracts contributed more to its antioxidant activity. This could be due to DPPH being a free radical that requires a hydrogen atom to become stable, while FRAP is a non-free radical and requires an electron transfer to be reduced from its vulnerable state to a more stable one (ferric to ferrous ion). Certain antioxidant compounds in the extract can kinetically react in different mechanisms towards the free radical or any other reactive forms. Probably the compounds in the extract are metal-deactivating antioxidants that could readily transfer electron to reduce the ferric to ferrous ion, and make it less reactive (Sethi *et al.*, 2020).

Multivariate data analysis

Figure 1 illustrates the score scatter plot for the OPLS model of all *M. charantia* fruit extracts analysed using GC-MS in correlation to the antioxidant activities. The UV scaling was employed in which the variation of the variables was scaled to the same units (Bujak *et al.*, 2016). The OPLS showed a maximum variation with the values of 34.5 and 50.2% for the OPLS components 1 and 2, respectively. The score scatter plot showed that the resulting clustered grouping was concurrent to its antioxidant activity and well discriminated along with OPLS component 1. The extracts with high antioxidant activity (100% and 80% ethanol:water extracts) were well distributed on the left side of OPLS component 1. The gradient shifting of the extract along OPLS component 1 to the left side was proportional to its antioxidant activity.
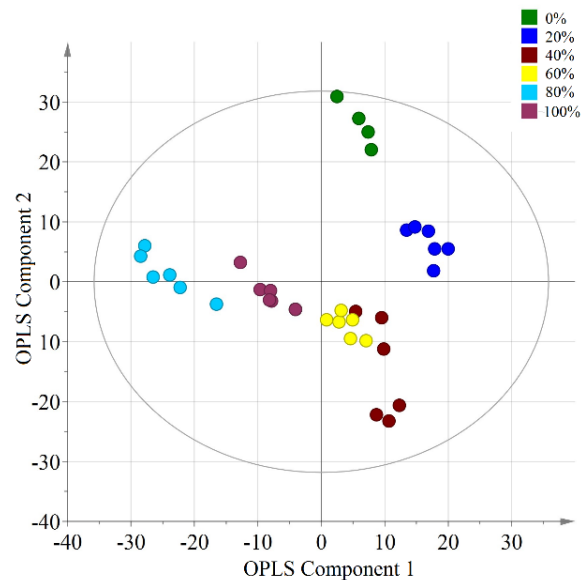

Figure 1. Score scatter plot for OPLS model of different extracts of *M. charantia* fruit (0, 20, 40, 60, 80, and 100% aqueous ethanolic extracts) analysed using GC-MS.

Multivariate data analysis is validated using the response to the permutation test. The permutation test estimates that the distributions of R^2Y and Q^2Y when the Y-vector is randomly permuted. Not only that, these distributions are compared with the values of R^2Y and Q^2Y for the actual model with non-permuted Y (Worley and Powers, 2016). Thus, by performing a permutation test, the reliability of the developed OPLS model can be determined. Figures 2 (A) and (B) show the permutation for the DPPH and FRAP models, respectively. The intercept values of R^2Y and Q^2Y for the DPPH model were found to be 0.306 and

-0.443 with 0.261 and -0.458, respectively, for the FRAP model. The models generated were considered valid as they satisfied the requirement for permutation test which is the intercept values for R^2Y and Q^2Y should be below 0.3 and -0.05, respectively (Kuhlisch and Pohnert, 2015).

Once a validated model was obtained, it was possible to identify the metabolites responsible for the sample discrimination. For this, the metabolites with values above 1.0 in a variable of importance were selected. Laterally, the column loading plot
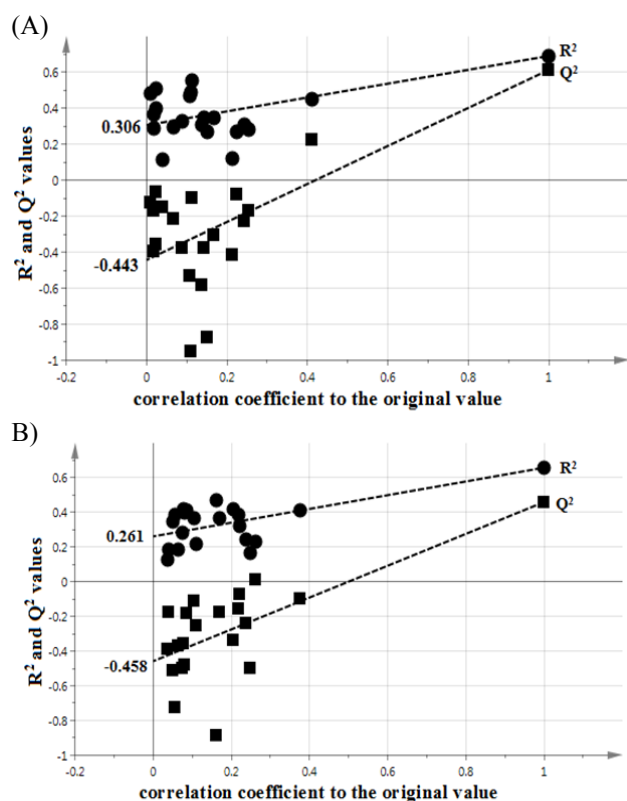
(A)

B)

Figure 2. Permutation test for OPLS model of GC-MS correlated to DPPH radical scavenging (A) and FRAP (B) activities.

from the OPLS model was used to determine the significant metabolites. It showed that the metabolites that fell below the negative probabilistic quotient (pq) were radical scavengers, responsible for the lower IC_{50} values in the DPPH antioxidant assay, and the low ferric reducing ability in the FRAP antioxidant assay. Conversely, the metabolites that fell under a positive pq were pro-oxidants. The column loading plot revealed the GC-MS retention times correlated to the antioxidant activities. The assignment of the antioxidants in each retention time was carried out by comparing the fragmentation pattern to the NIST database as tabulated in Table 2. The matched compounds with probability of more than 80% were assigned as the identified compounds. Gentiobiose, glucose, galactonic acid, palmitic acid, galactose, mannose, and fructose were the tentative metabolites responsible for the antioxidant activities of *M. charantia* extract.

Synergistic effect of suspected bioactive compounds

Compounds identified as antioxidants through the GCMS-based metabolite profiling of ethanol:water extracts of *M. charantia* fruit were previously unreported for their antioxidant potentials from any source or through any research study. Therefore, a confirmation on their antioxidant activities was required in the present work to confirm if true antioxidants had been identified through the GCMS-based metabolite profiling. Hence, each individually identified compound was tested through FRAP and DPPH radical scavenging activities assays, and the result is tabulated in Table 3. All the identified compounds were found to exhibit different degrees of antioxidant activities through both aforementioned assays. However, the antioxidant activities of all the identified compounds

Table 2. Metabolites identified in the extracts of *M. charantia* fruit*.

No	Retention time (min)	Area (%)	Probability (%)	Molecular weight (M⁺)	Tentative metabolite
1	25.08	27.11 ± 0.30	99	178.14	Galactonic acid
2	25.69	14.94 ± 0.56	90	180.16	Glucose
3	26.18	3.07 ± 0.07	87 - 91	180.16	Mannose
4	26.24	3.57 ± 0.11	91	180.16	Galactose
5	27.08	0.28 ± 0.02	87 - 91	180.16	Fructose
6	30.13	0.09 ± 0.00	93 - 99	256.42	Palmitic acid
7	47.09	0.16 ± 0.02	80 - 90	342.30	Gentiobiose

*Based on NIST 2014 (Gaithersburg, MD, USA) database.

on both *in vitro* tests were found to be weaker in comparison to the positive control, *i.e.,* ascorbic acid.

Table 3. DPPH and FRAP antioxidant analysis of individual analyte.

Analyte	DPPH (%)	FRAP (AAE µg of ascorbic acid/g)
Gentiobiose	16.34 ± 2.27 [bc]	1.78 ± 0.24 [c]
Glucose	6.60 ± 1.40 [d]	1.95 ± 0.11 [c]
Galactonic acid	13.50 ± 0.97 [c]	1.71 ± 0.04 [c]
Palmitic acid	20.72 ± 0.98 [b]	32.97 ± 0.80 [b]
Galactose	5.77 ± 1.46 [d]	2.08 ± 0.02 [c]
Mannose	16.61 ± 1.02 [bc]	2.45 ± 0.23 [c]
Fructose	16.99 ± 1.05 [bc]	2.20 ± 0.07 [c]
Ascorbic acid	99.70 ± 0.02 [a]	114.58 ± 1.73 [a]

Values are mean ± standard error mean (SEM) of six replicates ($n = 6$). Means in each column with different lowercase superscripts are significantly ($p < 0.05$) different as compared to standard. The assay concentration of the sample was 0.4 mg/mL for DPPH, and 0.2 mg/mL for FRAP.

This clearly indicated the presence of synergistic effect among the identified compounds and other compounds in the ethanol:water extract of *M. charantia* fruit. In many cases, the fractions and purified compounds were found to diminish in their bioactivities after separation or purification (Kuhlisch and Pohnert, 2015). Therefore, the ethanol:water extract of *M. charantia* fruit was needed to be spiked with the individually identified compounds to evaluate this synergistic effect.

The quantification of each identified compound was carried out to determine the amount of the identified antioxidants to be added to the *M. charantia* extract. To determine the synergistic effect, 80% ethanol:water extract of *M. charantia* fruit was spiked with each of the identified compounds responsible for the antioxidant effect. It was spiked with 2-, 4-, and 8-time the original amount of each identified compound in the extract. The outcome indicated that only the extract spiked with gentiobiose showed an increment of FRAP activity when the extract was spiked with a double or higher amount of gentiobiose. In comparison, the other compounds showed the increment of the activity by spiking the extract with eight times higher amount of the respective compounds.

Moreover, the increment of the DPPH radical scavenging activity could be noticed when the extract was spiked with double or higher of most of the identified compounds, except gentiobiose and galactose. The increment of the activity was detectable when gentiobiose and galactose were spiked four times or higher to the extract. Hence, the present work confirmed that the tentative compounds were correlated to the antioxidant activities by working synergistically with other compounds in the extract.

The GC-MS-based metabolomics have been shown as an effective tool to profile the metabolites and identify the antioxidants in *M. charantia* fruit ethanol:water extracts in the present work. Many sugars such as glucose, mannose, gentiobiose, and fructose have been detected as antioxidants in the extracts. However, no scientific report was found on the antioxidant activity of individual monosaccharide or disaccharide, although some researchers have found the relationship between the monosaccharide composition of polysaccharides and their antioxidant activities (Zhu *et al.,* 2019; Duan and Yu, 2019). The absence of antioxidant activity reports of these sugars was expected since these sugars are not as active as an antioxidant in the pure state, which was double-confirmed in the present work. The sugars work synergistically with other unknown compounds in the extracts, which was confirmed by spiking the individual sugars into the extract. This is one of the advantages of using metabolomics where it is able to trace the bioactive compounds in the extract without losing the bioactivity due to the separation of the compounds.

Other antioxidants that were detected by this approach in the present work were palmitic acid and galactonic acid. Although both compounds did not show antioxidant activities in the present work, they were still found to increase the antioxidant activities after spiking to the 80% ethanol:water extract of *M. charantia* fruit. This indicated the synergistic effect of other compounds in the extract. In addition, palmitic acid has been found to act on ROS-mediated cell proliferation and tumorigenesis (Wang *et al.,* 2011). It stimulates the hepatocyte proliferation through ROS-p38 MAPK/ERK-Akt cascade activation which interferes with the activation of Nrf2. The effect of ROS on signal transduction was found to be dose- and time-dependent. However, the role of palmitic acid as an antioxidant compound should be further reviewed since it promotes DNA damage in an insulin-secreting cell line, mainly human fibroblasts (Fernandes *et al.,* 2017).

Galactonic acid, which is also known as

sugar acid, plays a role in the antioxidative system and electron sink for photoprotection and cold tolerance in plants. The introduction of a galactonic acid precursor significantly increases the levels of the antioxidant metabolites glutathione and ascorbate (Broad *et al.,* 2020). Another study further supports this, reporting that glutathione and ascorbate protect labile macromolecules by scavenging hydrogen peroxidase and free radicals from oxidative stress produced by extreme amounts of herbicides, air pollutants, droughts, or temperature (Diaz-Vivancos *et al.,* 2015; Veljović-Jovanović *et al.,* 2017).

Conclusion

The present work determined the chemical constituents that are responsible for the antioxidant activity of *M. charantia* fruit using a GC-MS metabolomics approach. The 80% ethanol:water extract exhibited marked antioxidant activities based on DPPH and FRAP assays. The main potentially antioxidant compounds were gentiobiose, glucose, galactonic acid, palmitic acid, galactose, mannose, and fructose.

Acknowledgement

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