Malays. Appl. Biol. (2021) 50(1): 181–188

POLYPHENOLIC PROFILE AND ANTIOXIDANT ACTIVITIES OF FREEZE-DRIED MELON MANIS TERENGGANU PEEL EXTRACTS

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Accepted 27 May 2021, Published online 10 June 2021

ABSTRACT

Melon Manis Terengganu (MMT) also known as *Cucumis melo* var. Inodorus cv. Manis Terengganu 1 originates from Terengganu, Malaysia, is composed of 28–30% inedible peels and discarded as waste. Hence, this study aimed to quantify the polyphenol and flavonoid contents, identify polyphenolic compounds and evaluate the antioxidant activity of freeze-dried Melon Manis Terengganu (MMT) peel aqueous extract. The total phenolic and flavonoid contents were determined spectrophotometrically by gallic acid and quercetin standard curves, respectively. Whereas, antioxidant activity was explored by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis (3-ethylben-zothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay. Next, liquid chromatography-mass spectrometry (LC-MS) was applied for phenolic compounds identification. Results reported that the total phenolic content was 12.35 (0.59) µg GAE/mg while total flavonoid content was 2.01 (0.70) µg QE/mg. The IC_{50} of DPPH and ABTS assay were 27.74 (1.59) mg/mL and 4.87 (0.06) mg/mL, respectively. LC-MS results revealed the presence of polyphenolic compounds, such as kaempferol 3-(6''-sinapylglucosyl)-(1->2)-galactoside), isoorientin 7-O-(6'''-O-(E)-feruloyl)glucoside and isoscoparin 2''-(6-(E)-ferulylglucoside) in the sample extract. In summary, these findings served as preliminary data for further exploration on the potential application of freeze-dried MMT peel aqueous extract in the food and nutraceutical industry.

Key words: Antioxidant, freeze-drying, Melon Manis Terengganu peel, polyphenols

INTRODUCTION

Freeze-drying is also known as lyophilization in which water removal via sublimation from solid-state to vapor phase (Chua *et al*., 2019) under low temperature (–2°C to –50°C) and vacuum conditions to obtain dried products (Karam *et al*., 2016). Evidence suggested that freeze-drying is a preferable and widely used method to dry thermosensitive compounds, such as polyphenols, tocopherols, ascorbic acid, and carotenoids (Amaro *et al*., 2015) to obtain a nutritious functional food ingredient (Tumbas Šaponjac *et al*., 2016) by maintaining the quality (Suravanichnirachorn *et al*., 2018). This is because thermal degradation reactions were nearly prohibited, which results in the retention of physical, chemical, and functional properties of samples (Ceballos *et al*., 2012; Coklar *et al*., 2018). However, some drawbacks are associated with freeze-drying, such as it needs longer time compared to other methods due to low drying rate, lower yield, greater energy consumption, high operation and production cost, including loss of some volatile compounds (Karam *et al*., 2016; Tatar Turan *et al*., 2015).

Surprisingly, a study revealed that worldwide more than 500 Megaton (Mt) of fruits and vegetables peel were discarded as waste during food processing (Banerjee *et al*., 2017). It is well-known that most of

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these wastes consisted of nutrients and bioactive compounds which can enhance human health (Ganji *et al*., 2019). One fruit of interest is Melon Manis Terengganu (MMT), which is recognized as *Cucumis melo* var. Inodorus cv. Manis Terengganu 1 and credited with antioxidant capacity, as reported by Ong *et al.* (2019). This is attributed to the presence of bioactive compounds, such as vitamins, polyphenol, and cucurbitacins (Amaro *et al*., 2015; Petkova & Antova, 2015). Normally, MMT is peeled before consumption, which consists of 28% to 30% peel (Ong *et al*., 2021). Moreover, a study found that the MMT peel contained the highest epigallocatechin gallate (EGCG) concentration as compared to its flesh and seed (Ong *et al*., 2020). However, there is limited information about the polyphenolic profile and antioxidant capacity of MMT peel. Indeed, it is appealing to explore the novel utilization of MMT peel. Therefore, the present study aimed to quantify the polyphenol and flavonoid contents, as well as evaluate the antioxidant activities and phenolic profile of freeze-dried MMT peel aqueous extract.

MATERIALS AND METHODS

Materials

All chemicals and reagents used were of analytical grade. Ethanol, methanol, aluminum chloride, sodium acetate, Folin-Ciocalteau (F–C) phenol reagent were obtained from Merck (Darmstadt, Germany). Gallic acid and quercetin standards were purchased from Acros Organics (NJ, USA). Next, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis (3-ethylben-zothiazoline-6-sulfonic acid) diammonium salt (ABTS), Trolox standard, potassium persulfate, and sodium carbonate were obtained from Sigma-Aldrich (St Louis, USA. Diclofenac sodium was purchased from Sigma-Aldrich (St Louis, USA). Distilled water was purified using the Sartorius water purification system (Germany).

Preparation of freeze-dried MMT peel aqueous extract

MMT at uniform maturity stage (65 days after seeding) was collected from Mega Fertigation Farm, Kampung Telaga Papan, Setiu, in Terengganu. The peel was obtained in the form of a ± 0.5 cm thickness cut. The sample preparation, extraction, and freeze-drying process were performed according to the previous procedure described (Ong *et al*., 2020). Briefly, the MMT peel was dried and ground into powder form followed by water extraction and freeze-drying.

Antioxidant activity

The total phenolic content (TPC), total flavonoid content (TFC), DPPH, and ABTS assay were carried out following the procedure as previously outlined by Chang *et al*. (2002), Chatatikun and Chiabchalard (2013), Elisha *et al.* (2016) and Wang *et al.* (2018) respectively. All analyses were conducted three times.

Liquid chromatography-mass spectrometry (LC-MS)

The freeze-dried MMT peel aqueous extract was analyzed by using Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-time-of-flight (TOF) mass spectrometer with dual electrospray ionization (ESI) source. The HPLC separation was carried out on an Agilent Zorbax Eclipse XDB-C18, Narrow-Bore column (2.1 mm × 150 mm, 3.5 µm) operated at 25°C. The mobile phase, which consisted of a 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B), was delivered at a flow rate of 0.5 mL/min under a gradient program: 5% (B) from 0 to 5 min, 5–100% (B) from 5 to 20 min and 100% (B) from 20 to 25 min. The post run time was 5 minutes. The sample injection volume was 1 µL with autosampler temperature of 4°C.

MS conceded in negative ionization mode within a mass range of m/z 100-3200. Nitrogen was used as drying and collision gas. The heated capillary temperature was set to 300°C and nebulizer pressure to 45 psi. The drying gas flow rate was 10 L/min. VCap, fragmentor, skimmer and octapole RF peak voltages were set to 3500V, 125V, 65V and 750V, respectively, in the ion source parameters. The chromatographic and mass spectrometric analyses, including the prediction of chemical formula and exact mass calculation were performed by using Agilent Mass Hunter Qualitative Analysis version B.07.00.

Data analysis

The research data were analyzed by using IBM SPSS for Windows Version 21.0. The data were assessed by descriptive analysis and presented as mean and standard deviation.

RESULTS AND DISCUSSION

As can be seen from Table 1, TPC of MMT peel aqueous extract was 12.35 (0.59) µg GAE/mg identified via a linear gallic acid standard curve ($y = 0.0111x + 0.0868$, $R^2 = 0.9493$). Meanwhile, quercetin standard curve with $y = 0.0089x + 0.016$ and $R^2 = 0.9914$ was used to determine TFC of 2.01 (0.70) µg QE/mg. TPC of freeze-dried MMT peel

Table 1. Polyphenolic content and antioxidant activity of freeze-dried MMT peel aqueous extract (n=3)

Antioxidant activities	Readings
Total phenolic content (μg GAE/mg)	12.35 (0.59)
Total flavonoid content (μg QE/mg)	2.01 (0.70)
1,1-diphenyl-2-picrylhydrazyl (DPPH) assay IC_{50} (mg/mL)	27.74 (1.59)
2,2-azino-bis (3-ethylben- zothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay IC_{50} (mg/mL)	4.87 (0.06)

Data are reported as mean (SD).

aqueous extract was greater as compared to previous studies (0.14 ± 0.01 μg GAE/mg in *Cucumis melo* L. var. reticulatus by Fundo *et al*., 2018; 0.69 ± 0.06 to 2.96 ± 0.12 μg GAE/mg by Ganji *et al.*, 2019; 4.70 ± 0.23 μg GAE/mg in *Cucumis melo* L. var. reticulatus by Ismail *et al*., 2010; 3.32 ± 0.00 μg GAE/mg in *Cucumis melo* L. cv. maazoun by Mallek-Ayadi *et al*., 2016; 8.47 ± 0.21 μg GAE/mg in *Cucumis melo* var. cantalupensis by Marwa & Hany, 2016; 2.27 ± 0.42 μg GAE/mg by Morais *et al*., 2015; 1.11 ± 0.15 μg GAE/mg in *Cucumis melo* L. var. reticulatus by Rolim *et al.*, 2018; 0.23 ± 0.01 μg GAE/mg in muskmelon by Singh *et al.*, 2016) and two-fold lower than that of a study byVella *et al*. (2019) which reported 25.48 μg GAE/mg in *Cucumis melo* L. var. reticulatus Meanwhile, the TFC of freeze-dried MMT peel aqueous extract was higher than previous studies by three to 30 folds (0.95 ± 0.00 μg QE/mg in *Cucumis melo* L. cv. maazoun by Mallek-Ayadi *et al*., 2016; 2.00 ± 0.60 μg QE/mg by Morais *et al.*, 2015; 0.06 ± 0.00 μg QE/mg in muskmelon by Singh *et al.*, 2016). The discrepancies of polyphenolic contents could be attributed to several factors such as maturation level during harvesting (Rodríguez-Pérez *et al*., 2013), edaphic factors (soil conditions, sunlight exposure, and rainfall) (Rolim *et al*., 2019), growing region (Jakobek *et al*., 2013), growing seasons, genotype or cultivar (Tadmor *et al*., 2010), cultural practices (Miletić *et al*., 2012) as well as ambient conditions of previous harvests, post-harvest, and processing (Pandey & Rizvi, 2009). Besides, the gap could be due to the differences in drying and extraction conditions. In this study, the possible factors contributed to these differences might be due to the varied types of melon, drying and extraction parameters.

Next, the antioxidant activity of MMT peel aqueous extract was determined by DPPH and ABTS assay in terms of IC_{50} with 27.74 (1.59) mg/mL and 4.87 (0.06) mg/mL, respectively. Nevertheless, the IC_{50} remains lower as compared to the standard (quercetin), with an IC_{50} of 0.02 (0.00) mg/mL in DPPH assay, and standard (Trolox) with an IC_{50} of 0.06 (0.00) mg/mL in ABTS assay. Otherwise, the MMT peel aqueous extract scavenge DPPH and ABTS radicals in a dose-dependent manner, with inhibition being greater than 81% at 50 mg/mL (Figure 1) and 86% at 6 mg/mL (Figure 2), respectively. The IC_{50} of the DPPH assay in this study was lower than other studies (9.58 ± 0.37 mg/mL in *Cucumis melo* L. var. *reticulatus* by Ismail *et al*., 2010; 0.19 ± 0.00 mg/mL by Morais *et al.*, 2015; 0.32 to 0.50 mg/mL in rockmelon by Norrizah *et al*., 2012; 6.65 mg/mL in *Cucumis melo* L. var. *reticulatus* by Vella *et al*., 2019; 0.0046 to 0.0053 mg/mL by Wakid & Harun, 2020). Normally, the inhibition of $ABTS^{\cdot+}$ activity is usually used to confirm with $DPPH^{\cdot}$ scavenging capacity since both are similar in their antioxidant mechanisms in which both radicals can receive electrons and $H^{\cdot}$ from the antioxidant samples (Singh *et al*., 2016; Santos-Sánchez *et al*., 2019). In this study, the IC_{50} of ABTS was lower than that of DPPH. One possible explanation for this discrepancy could be attributed to the difference in the antioxidant action of the assay (Nayak *et al*., 2015). The reaction of antioxidants with ABTS radical is much faster than DPPH radical. The slow and fast reactions of antioxidants with ABTS radical can attain a steady-state within 6 min. Meanwhile, the fast, intermediate, and slow reactions of antioxidants with DPPH radical need 0 to 5 min, 5 to 30 min, and more than 30 min, respectively, to attain the steady-state. In this study, the incubation time covered both slow and fast reactions in ABTS assay, but only fast and intermediate reactions in DPPH assay. Indirectly, the results implied that the freeze-dried MMT peel aqueous extract may contain considerable slow-acting antioxidants towards DPPH radical (Yang *et al*., 2011).

Freeze-drying is one of the most effective methods to preserve thermo-labile bioactive compounds better as compared to other drying methods (Zori *et al*., 2016; Coklar *et al*., 2018). Extensive research demonstrated that greater total phenolic, tannin, and flavonoid contents were found in samples subjected to freeze-drying such as in pomegranate peel (Mphahlele *et al*., 2016), pomegranate rind (Calín-Sánchez *et al*., 2013), and persimmon (Karaman *et al*., 2014). Phenolics and flavonoids are considered as plants' secondary metabolites which take part in a complex system of antioxidant defense via free radical scavenging

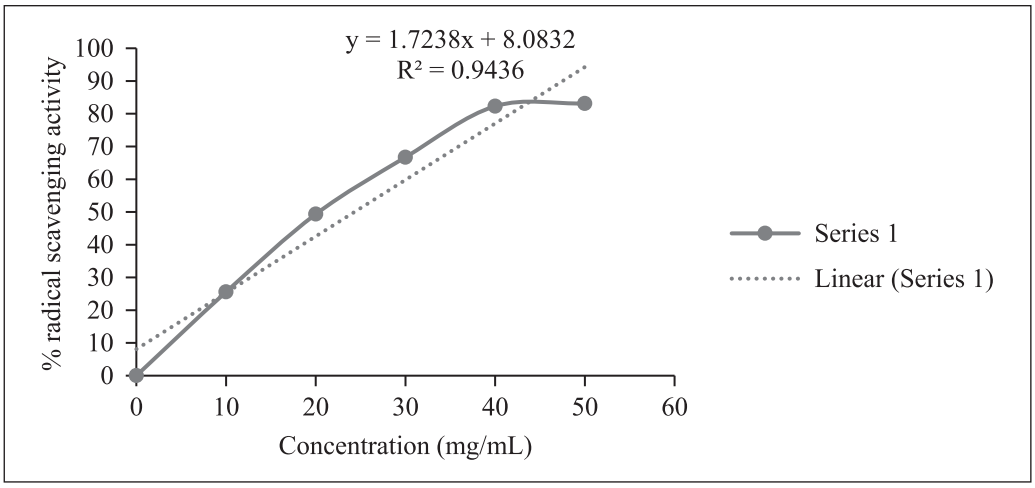

Fig. 1. DPPH radical scavenging activity of freeze-dried MMT peel aqueous extract.

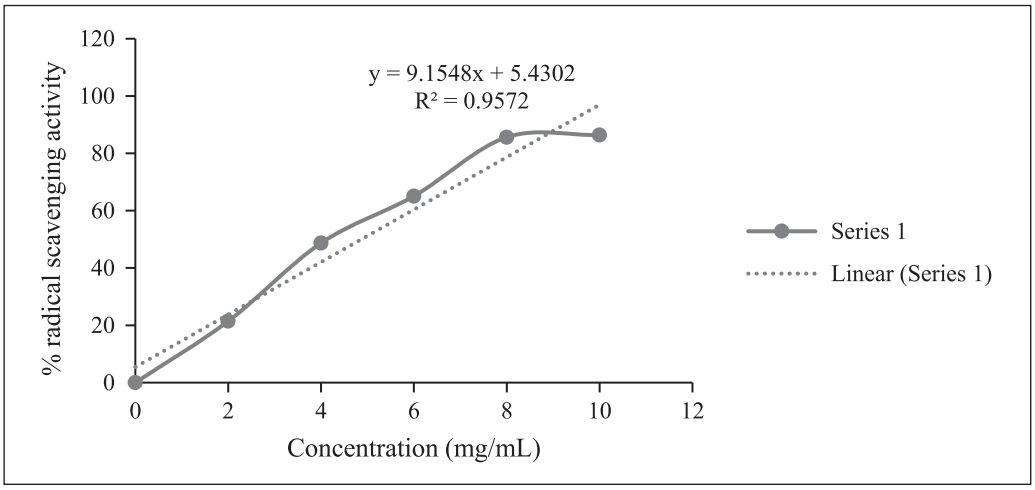

Fig. 2. ABTS radical scavenging activity of freeze-dried MMT peel aqueous extract.

activity, chelation of transition metals, suppression of pro-oxidative enzymes (Bouaziz *et al*., 2020), and synergistic interaction with other antioxidants (Andreicut *et al*., 2018). Literature reports that the shikimate (shikimic acid) biosynthetic pathway is involved in the generation of the aromatic melon compounds which seem to be responsible for the antioxidant properties of the melon peel (Ismail *et al*., 2010). Polyphenols are also recognized as potent antioxidant compounds with higher free radical scavenging activity as compared to vitamin C and vitamin E (Coklar *et al*., 2018). It is also well established that phenolic compounds, particularly flavonoids, are responsible for free radical scavenging activity. However, it should be noted that antioxidant activity does not depend solely on phenolic content, but is also influenced by other compounds, such as ascorbic acid, flavonoid, and carotenoid (Wulandari *et al*., 2017).

As for the identification of phenolic compounds by LC-MS, different m/z values were detected (Table 2) with three compounds deduced based on their molecular structures (Figure 3). The identified phenolic compounds were classified into flavonol group (kaempferol 3-(6''-sinapylglucosyl)-(1->2)-galactoside) and flavone group (isoorientin 7-O-(6'''-O-(E)-feruloyl)glucoside and isoscoparin 2''-(6-(E)-ferulylglucoside)). These bioactive compounds belong to derivatives of kaempferol, isoorientin, and isoscoparin. A previous study also demonstrated that the cantaloupe melon peel contained kaempferol (Vella *et al*., 2019). Kaempferol exhibited various beneficial health effects, such as antioxidant (Arif *et al*., 2018; Wang *et al*., 2018), anti-inflammatory (Wang *et al*., 2018; Nascimento *et al*., 2017), anti-diabetic (Li *et al*., 2017), and anti-arthritic (Zhuang *et al*., 2017; Huang *et al*., 2018) properties. Next, isoorientin also had the potential to act as an antioxidant (Yuan *et al*., 2016) and anti-inflammatory (Anilkumar *et al*., 2017) agent based on previously reported studies. Last but not least, previous research also claimed isoscoparin possesses antioxidant

Table 2. LC-MS of freeze-dried MMT peel aqueous extract

Retention time (min)	m/z $[M-H]^-$	Molecular formula	Compound identification
8.555	815.2054	C38 H40 O20	Kaempferol 3-(6''-sinapylglucosyl)-(1->2)-galactoside
8.802	786.2022	C37 H38 O19	Isoorientin 7-O-(6'''-O-(E)-feruloyl)glucoside
8.999	799.2104	C38 H40 O19	Isoscoparin 2''-(6-(E)-ferulylglucoside)

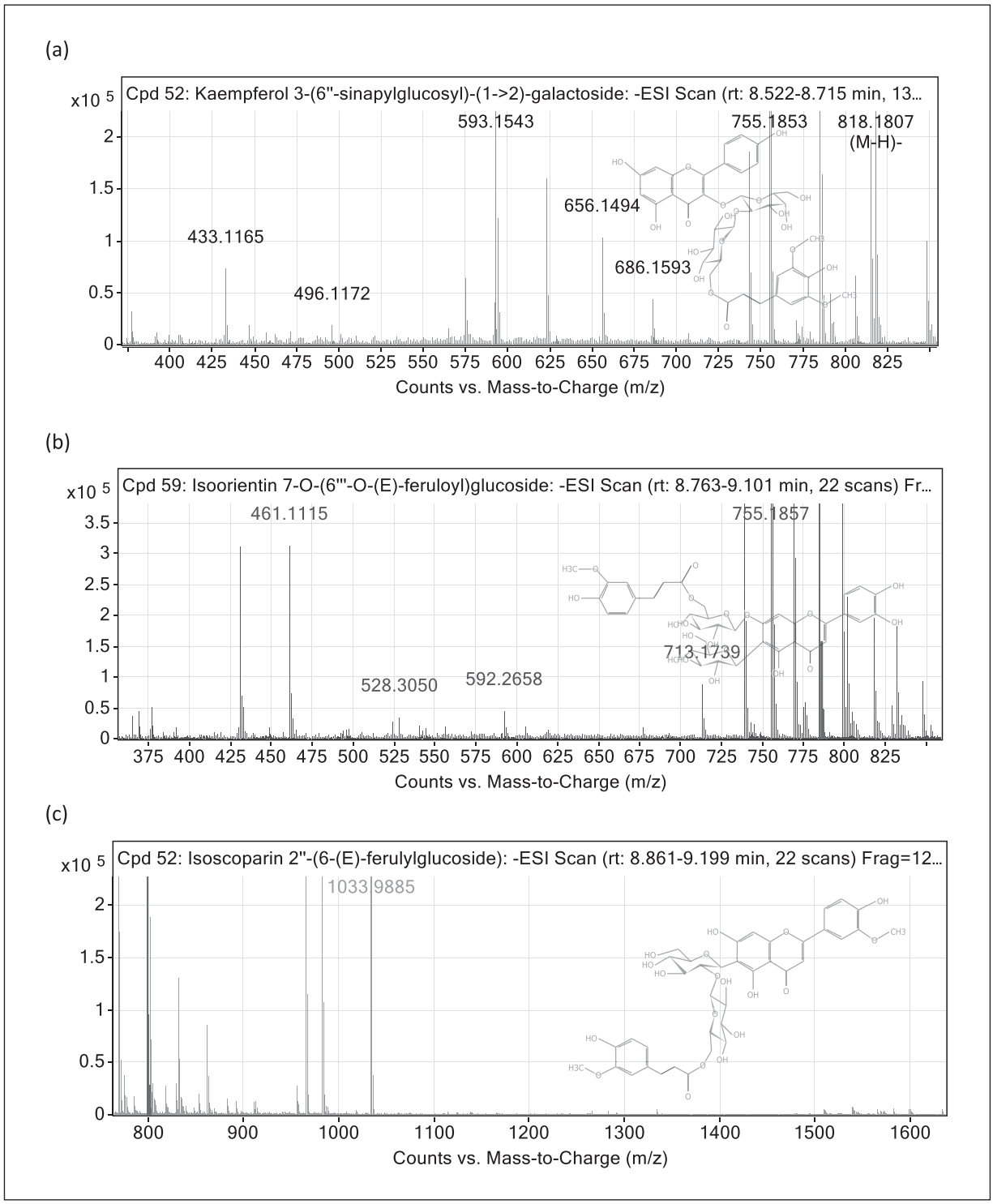

Fig. 3. MS spectrum of a) kaempferol 3-(6''-sinapylglucosyl)-(1->2)-galactoside b) isoorientin 7-O-(6'''-O-(E)-feruloyl)glucoside and c) isoscoparin 2''-(6-(E)-ferulylglucoside).

and anti-inflammatory capacity (Yang *et al*., 2016). These properties are beneficial to osteoarthritis, as reported by a scoping review (Ong *et al*., 2020). To conclude, the antioxidant properties showed by the freeze-dried MMT peel aqueous extract in this study could be attributed to the presence of these bioactive compounds.

CONCLUSION

The freeze-dried MMT peel aqueous extract contains antioxidant properties with the presence of polyphenolic compounds. These findings served as preliminary data for further exploration on the potential application of freeze-dried MMT peel aqueous extract in the food and nutraceutical industry. Further studies on MMT peel aqueous extract are warranted to identify the exact mechanism of action for its antioxidant properties.

ACKNOWLEDGEMENTS

This study was funded by the Ministry of Education, Malaysia under the Fundamental Research Grant Scheme (FRGS/1/2018/TK02/UNISZA/03/1). The authors would like to extend their gratitude to the LCMS Lab located in Monash University Malaysia for assisting LCMS. The authors are indebted to everyone who has contributed to this research.

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