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Determination of Vitamins, Minerals, Heavy Metals and Anti-Inflammatory Activity of Melon Manis Terengganu Peel

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Melon Manis Terengganu (MMT) is usually peeled prior to consumption and it consists of 28% to 30% peel. Several elements of the MMT peel have been identified to benefit the human health, while some are known for their deleterious effect on human. As such, this study had determined the concentration of vitamins, minerals, and heavy metals, apart from assessing the anti-inflammatory capacity of MMT peel. Vitamins A, D, E, K, and C were quantified using Association of Official Analytical Chemists (AOAC) official methods. Next, the samples were acid digested using microwave system, followed by minerals and heavy metals content quantification by inductively coupled plasma-optical emission spectrometry (ICP-OES). Anti-inflammatory activity was examined using protein denaturation inhibition and human red blood cell (HRBC) membrane stabilization assay. The results demonstrated that vitamin C had the highest content in MMT peel with 5.60 (0.04) mg/100g. Meanwhile, magnesium (458.72 (6.72) mg/100g) was the predominant mineral found in MMT peel with heavy metals concentration below permissible limit. The half maximal inhibitory concentration (IC_{50}) of protein denaturation assay and HRBC membrane stabilization assay were 5.75 (0.14) mg/mL and 35.70 (0.37) mg/mL, respectively. In conclusion, the findings can valorize the potential application of MMT peel as a dietetic and nutritional product.

Keywords: anti-inflammatory, heavy metals, Melon Manis Terengganu peel, minerals, vitamins

INTRODUCTION

Functional foods are one of the most fascinating scopes of research and innovation within the food domain, as evidenced by the raising amount of empirical articles related to this area since year 2007 (Corbo, Bevilacqua, Petruzzi, Casanova, & Sinigaglia 2014). At present, consumers are more concerned about their health by focusing on their lifestyle and dietary intake. Interestingly, green consumerism has garnered the interest of the universal society by stating preference for natural products over synthetic additives (Azima, Suryanti, & Syukri 2018). Many plant foods or bioactive compounds acquired from plants have been explored for their contribution in disease prevention and health (Pushpangadan et al. 2014). Some of the bioactive compounds are vitamins, carotenoids, phenolic compounds (phenolic acids, flavonoids, and phytoestrogens), tocotrienols, prebiotics, organosulfur compounds, phytosterols, and phytostanols, as well as non-digestible carbohydrates (dietary fibers) (da Silva & Jorge 2014; Mallek-Ayadi, Bahloul, & Kechaou 2016). Since fruits and vegetables are natural sources of active components, their consumption has acquired popularity (Sudibyo 2018).

The Cucurbitaceae family or cucurbits is a group of fruits that has been vastly investigated by researchers due to its various biological activities (Ong, Sakinah, Shahril, & Norshazila 2019). Melon Manis Terengganu (MMT) or *Cucumis melo* var. Inodorus cv. Manis Terengganu 1 originates from the Cucurbitaceae family. While processing MMT, a large amount of by-products, such as peels, rinds, seeds, and unused flesh, are produced. These by-products are usually discarded with no further use and may lead to serious environmental issue that demands immediate action (Mallek-Ayadi, Bahloul, & Kechaou 2017). Agro-industrial residues contain good natural sources of bioactive ingredients, like vitamins, minerals, pigments, and antioxidants, which have the potential to serve as food additives or nutraceuticals (Watson 2019). The MMT peel accounts for 28% to 30% of the whole fruit. The use of fruit peels has caught the attention of researchers as these peels demonstrate superior biological activities, when compared to other fruit parts. This notion is supported by a few studies, wherein higher amount of bioactive compounds found in the peel exert beneficial health effects, such as anti-inflammatory and anti-arthritic activity (Ong, Sakinah, Shahril, Norshazila, & Sia 2020). However, heavy metals found in peel due to use of pesticides is a health concern. To the best of the authors' knowledge, so far information about the nutritional elements and biological activities of MMT peel is in scarcity. Hence, this study identified the vitamins, minerals, and heavy metals contents of MMT peel, apart from investigating its anti-inflammatory activity.

MATERIALS AND METHODS

Reagents and materials

All chemicals and reagents used were of analytical grade.

Sample collection and extract preparation

The sample preparation, extraction, and freeze-drying process were performed by adhering to those prescribed in previous study (Ong et al. 2020). Briefly, 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 ± 0.5 cm thickness cut, which had been dried and ground into powder form. Then, the MMT peel was subjected to water extraction and freeze-drying.

Vitamins quantification

Vitamins A, E, K, and C were determined using AOAC official methods 960.45, 971.30, 999.15, and 967.21, respectively. Vitamin D was identified using high performance liquid chromatography (HPLC).

Minerals and heavy metals quantification

Firstly, all plastic and glassware containers were washed properly with detergent, cleaned under tap water, cleansed with deionized water, and immersed in nitric acid (10% v/v) overnight. These containers were cleansed a few times using deionized water and dried in cabinet dryer prior to use (Hong et al. 2019).

Instrumentation

The microwave system used in sample digestion was Multiwave Go, Anton Paar (GmbH, Graz, Austria), which was equipped with 12 high-pressure polytetrafluoroethylene vessels known as digestion tubes. The ICP-OES used was an iCAP 7000 series with ASX-520 auto sampler.

Microwave samples digestion

The ground samples were underwent microwave-assisted acid digestion based on the method described with slight modifications (Hong et al. 2019). First, each sample (0.5 g) was accurately weighed straight away into microwave digestion vessels and added with 9.0 ml of concentrated nitric acid (65%). The digestion procedure was as shown: (1) heating to 180 °C for 0-20 min, (2) 180 °C for 10 min, and (3) 70 °C for 10 min to cool. Following cooling, the constituents of the tubes were shifted to 25 ml polypropylene volumetric flask and diluted to 25 ml with deionized water for subsequent ICP-OES analysis.

ICP-OES conditions

The operating circumstances of the ICP device was established based on the manufacturer recommendations, as follows: radio frequency applied power (1.4 kW), argon plasma (10 L/min), auxiliary (0.5 L/min), and nebulizer (0.4 L/min) gas flow rates. The selected emission lines were: calcium (422.673 nm), magnesium (285.213 nm), zinc (202.548 nm), iron (259.940 nm), selenium (206.279 nm), copper (324.754 nm), manganese (257.610 nm), cadmium (228.802 nm), cobalt (238.892 nm), lead (220.353 nm), nickel (231.604 nm), and chromium (359.349 nm).

Anti-inflammatory activity

The anti-inflammatory activity of MMT peel was assessed via inhibition of protein denaturation and human red blood cell (HRBC) membrane stabilization assay according to the procedure described previously by Kariawasam, Pathirana, Ratnasooriya, and Handunnetti (2017) and Adnan et al. (2019), respectively.

Data analysis

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

RESULTS AND DISCUSSION

Table 1 displays an overview on the vitamins, minerals, and anti-inflammatory activity of MMT peel. Vitamin C displayed the highest content for vitamins category in MMT peel with 5.60 (0.35) mg/100g. In this study, β-carotene (0.33 mg/100g) and vitamin C (5.60 mg/100g) contents in MMT peel were two-fold higher than those found in watermelon rind (0.17 mg/100g and 2.93 mg/100g respectively) (Johnson et al. 2013). Vitamin C is an antioxidant and vital for collagen formation (Li & Schellhorn 2007). β-carotene is a precursor of vitamin A with antioxidant, anti-cancer, and anti-aging properties, besides protecting the retina of the human eye (Jang, Yoo, & Nam 2016). Vitamin A, D, E and K are fat soluble vitamins with anti-inflammatory or anti-arthritic effects. Vitamin A responsible in cartilage and skeletal formation (Zheng, Liang, Li, & Tu 2018), while vitamin D hinders inflammation and cytokine production (Shen et al. 2013). Additionally, vitamin D acts as a regulator of bone metabolism and calcium homeostasis via vitamin D receptors (Mabey & Honsawek 2015). Vitamin E is an antioxidant that enhances chondrocyte growth, prevents cartilage degradation, and boosts anti-inflammatory activity (Suantawee et al. 2013). Vitamin K engages in bone and cartilage mineralization (Zheng et al. 2018). The RNI for both male and female Malaysian adults are 600 µg, 15 µg, 7.5-10 mg, 55-65 µg, and 70 mg per day for vitamins A, D, E, K, and C, respectively (RNI *for Malaysia* 2017). RNI fulfilment of 55%, 3867%, 4-5% and 123-145% and 8% for vitamins A, D, E, K, and C, respectively was observed in 100 g of MMT peel.

Next, the highest concentration of minerals present in MMT peel was magnesium (458.72 (6.72) mg/100g), followed by calcium, iron, zinc, manganese, copper, and selenium. Minerals refer to inorganic materials present in soil or water and absorbed by plants or consumed by animals. Minerals are required to maintain proper body function and good human health (Sajib, Hoque, & Yeasmin 2014). In this study, the dominant mineral is magnesium, which is in disagreement with other studies that found calcium with the highest concentration in melon peel (Mallek-Ayadi et al. 2016; Morais et al. 2017). This discrepancy is attributed to the variation in terms of species and cultivation method. Calcium and magnesium are macro-minerals. The literature depicts that calcium can relieve inflammation-associated bone resorption (Klein 2018). Meanwhile, magnesium works with calcium to support healthy bones (Mallek-Ayadi et al. 2017). Magnesium can reduce low-grade systemic inflammation in chronic diseases (Nielsen 2018). Referring to the Recommended Nutrient Intake (RNI), Malaysian adults require 1000-1200 mg of calcium and 320-420 mg of magnesium daily (RNI *for Malaysia* 2017). In this study, 100 g of MMT peel accounts for 31-37% of RNI for calcium and 109-143% of RNI for magnesium.

Zinc, selenium, iron, manganese, and copper refer to trace elements or micro-minerals. They are antioxidant minerals that can significantly delay or prevent substrate oxidation when present in lower concentration than that of the substrate, thus functioning as antioxidant (Sajib et al. 2014). Zinc exhibits antioxidant and anti-inflammatory capacity by inhibiting oxidative stress and pro-inflammatory cytokines (Gammoh & Rink 2017). Selenium modulates excess immune responses and chronic inflammation (Huang, Rose, & Hoffmann 2012). A recent research reported that selenium can regulate cartilage homeostasis (Kang et al. 2020). Iron is involved in various physiological mechanisms, like oxygen transport, deoxyribonucleic acid (DNA) generation, and electron transport.

Table 1: Vitamins, minerals and anti-inflammatory activity of MMT peel (n = 3)

Vitamins	Amount (mg/100g)
Vitamin A (β-carotene)	0.33 (0.00)
Vitamin D	0.58 (0.01)
Vitamin E (α-tocopherol)	0.40 (0.00)
Vitamin K	0.08 (0.01)
Vitamin C	5.60 (0.35)
Minerals	**Amount (mg/100g)**
Calcium	366.32 (12.68)
Magnesium	458.72 (6.72)
Zinc	2.29 (0.07)
Selenium	0.31 (0.07)
Iron	7.81 (0.21)
Copper	0.42 (0.02)
Manganese	2.40 (0.08)
Anti-inflammatory activities	**Readings (mg/mL)**
Inhibition of protein denaturation assay IC_{50}	5.75 (0.14)
Human red blood cell membrane stabilization assay IC_{50}	35.70 (0.37)

Data are reported as mean (SD).

Table 2: Heavy metals content of MMT peel with regard to WHO/FAO permissible value (n = 3)

Heavy metals	WHO/FAO permissible value (mg/kg)	MMT peel (mg/kg)
Cadmium	0.2	0.05 (0.04)
Chromium	1.2	0.30 (0.05)
Lead	0.5	0.38 (0.12)
Nickel	0.5	0.15 (0.05)
Cobalt	2.0	0.23 (0.13)

Data are reported as mean (SD).

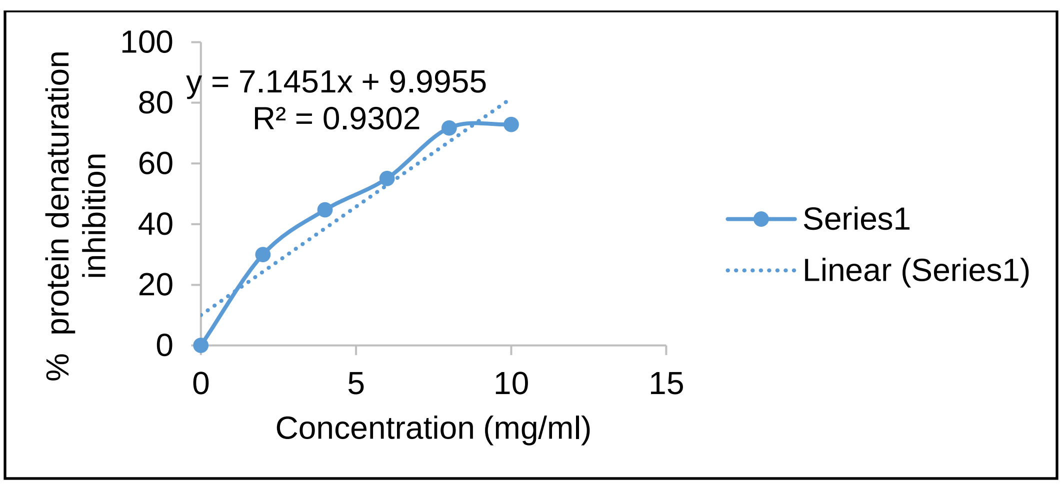

Figure 1: Inhibition of protein denaturation of MMT peel

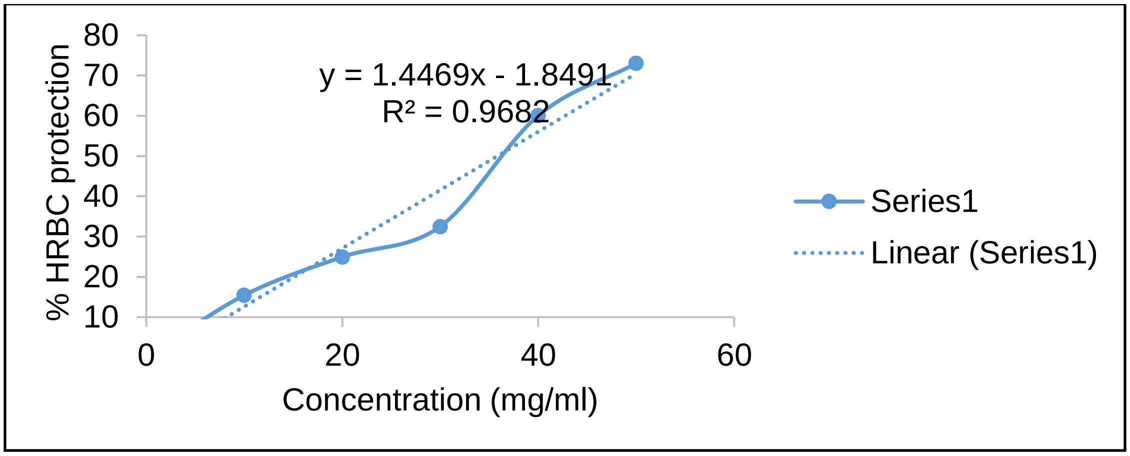

Figure 2: HRBC membrane stabilization of MMT peel

The level of iron in the body must be closely regulated as excessive amount may cause tissue damage attributed to the free radicals production (Abbaspour, Hurrell, & Kelishadi 2014). These trace elements are a cofactor of antioxidant enzymes. For instance, zinc, copper, and manganese are components for superoxide dismutase in both cytoplasm and mitochondria. Iron and selenium are constituents for catalase and glutathione peroxidase, respectively (Sajib et al. 2014). The RNI for zinc, selenium, iron, manganese, and copper in both men and women are 4.3-6.6 mg/day, 23-32 µg/day, 11 to 29 mg/day, 1.8-2.3 mg/day, and 900 µg/day respectively (RNI *for Malaysia* 2017). 100 g of MMT peel in the present study achieved 35-53%, 969-1348%, 27-71%, 104-133% and 46% of RNI for zinc, selenium, iron, manganese, and copper respectively.

Referring to Table 2, the heavy metals present in MMT peel was low in concentration and fell within the World Health Organization/Food and Agriculture Organization (WHO/FAO) permissible value (WHO 1999). Heavy metals, like cadmium, chromium, lead, nickel, and cobalt, are commonly found in pesticides (Wallace 2015), thus hazardous to individuals and other animals especially when the content level exceeds the permissible limit (Mansourri & Madani 2016). Another study demonstrated that the permissible safe threshold of heavy metals in food samples are related with reduced health threats in human (Sobhanardakani, Tayebi, & Hosseini 2018). Turning to this study, since the content of heavy metals is lower than the WHO/FAO permissible value, MMT peel does not trigger any safety or health concern to consumers under normal circumstances.

The *in vitro* anti-inflammatory activity of MMT peel was examined against egg albumin denaturation and HRBC membrane stabilization (see results in Table 1). It revealed that the anti-inflammatory activity of MMT peel in both assays increased with elevated concentration (Figures 1 and 2). Nevertheless, the effect of MMT peel with IC_{50} of 5.75 (0.14) mg/mL was low, when compared to standard (diclofenac sodium) with IC_{50} of 0.64 (0.01) mg/mL in egg albumin denaturation assay. Similarly, IC_{50} of HRBC membrane stabilization assay was 35.70 (0.37) mg/mL in the sample, which was lower than standard (diclofenac sodium) (0.61 (0.00) mg/mL). In the present study, the IC_{50} for both inhibition of egg albumin denaturation (5.75 mg/mL) and HRBC membrane stabilization (35.70 mg/mL) assays were higher compared to a study on whole *Cucumis melo* L. fruit by Singh & Devi (2020) which reported IC_{50} of 300 µg/ml and 260 µg/ml respectively. This implied that MMT is a less potent anti-inflammatory agent compared to the study by Singh & Devi (2020).

Inflammation is a complicated mechanism interrelated with the response of body tissues toward infection, irritation, and other injuries. Targeting enzymes that are responsible for the production of mediators to induce or intensify inflammatory process may serve as a basic for therapeutic treatment development, especially anti-inflammatory and anti-arthritis drug (Caesar & Cech 2019). Inhibition of egg albumin denaturation test is a commonly utilized, approved, sensitive, fast, and reliable method to explore in vitro anti-inflammatory activity of natural products (Kariawasam et al. 2017). The principle of this test is the destruction of egg whites proteins that cause auto-antigens generation related to type III hypersensitivity reaction, which results in inflammation (Agrawal & Paridhavi 2007; Kariawasam et al. 2017). The inflammation is closely related to several disorders, such as arthritis, diabetes, and cancer (Uttra & Alamgeer 2017). Protein denaturation is implicated by loss of secondary and tertiary structures that change from soluble to insoluble form. This ends up with loss of biological function of protein molecules (Sangeetha & Vidhya 2016).

Protein denaturation indicates possible changes in hydrogen, electrostatic, disulphide, and hydrophobic linkage (Gautam, Sharma, & Sharma 2013) induced by various chemical and physical sources, including acid or base, alcohol, acetone, salts of heavy metals and colorants, heat, light, and pressure (Sangeetha & Vidhya 2016). Higher degree of denaturation process inhibition implies greater anti-inflammation properties. The heating temperature is slowly increased from 37 °C to 70 °C, rather than instant heating to minimize generation of uneven lumps attributed to protein coagulation caused by water molecules evaporation from albumin protein and heat destruction of egg protein (Thereza, Gomes, Helena, & Pelegrine 2012). The specific mechanism where the extract exerts its anti-denaturation properties in the heat-stimulated protein denaturation is obscure currently and is yet to be investigated further. It might be due to the interaction of phenolic compounds with aliphatic area surrounding the lysine residue on the egg white protein. It could also be due to suppression of lysosomal components of neutrophils liberation at the inflammation area (Govindappa, Naga Sravya, Poojashri, Sadananda, & Chandrappa 2011).

The HRBC membrane stabilization is a well-established method to investigate anti-inflammatory and anti-arthritic activities (Gautam et al. 2013). Hemoglobin absorbance is measured in this assay since it is released as a result of HRBC membrane lysis attributed to less membrane stabilization (Sangeetha & Vidhya 2016). This method was chosen as the HRBC membrane is equivalent to the lysosomal membrane. Thus, stabilization of HRBC directly indicates lysosomal membrane stabilization (Kumar, Bhat, Kumar, Khan, & Chashoo 2012). Inhibition of HRBC hemolysis is closely associated with inflammatory process Lysosomal membrane stabilization can prevent lysis. Therefore, its stabilization is crucial in reducing inflammatory response by suppressing the liberation of lysosomal components from activated neutrophils, like bactericidal enzymes and proteases, as well as phospholipase A2 that can further induce tissue inflammation and injury upon extracellular liberation (Alamgeer, Umme Habiba Hasan & Rasoo 2015).

Distilled water was used to induce HRBC hemolysis that can liberate inflammatory mediators which results in tissue damage and chronic inflammation (Serhan 2009). Addition of 1.5 ml of distilled water can reduce osmotic pressure in the solution outside the HRBC (hypotonic). The HRBC remains comparable to 0.9% NaCl solution. Hence, water from outside of the HRBC will diffuse into the HRBC across the HRBC semi-permeable membrane against the concentration gradient (Adnan et al. 2019). The HRBC becomes swollen and consequently leads to rupture and hemolysis (Kumar et al. 2012). The specific mechanism of this membrane stabilization is still need to be explored, but it might be explained by the membrane expansion or cell shrinkage, as well as its interaction with membrane protein (Sangeetha & Vidhya 2016). Hypotonicity-prompted hemolysis may be caused by cell expansion attributed to osmotic entry of extracellular electrolyte and liquids constituents. The sample may suppress this process, thus inducing or enhancing the flow out of these extracellular constituents (Kumar et al. 2012).

CONCLUSION

From the nutritional perspective, MMT peel contains minerals and vitamins, which contributes to the daily requirement with anti-inflammatory capacity. The MMT peel, within the context of this study, does not cause health hazard amongst consumers due to the acceptable safe level of heavy metals concentration. Overall, these findings provide fundamental data to enhance the sitological value of MMT peel. Subsequently, it can valorize the potential application of MMT peel as a dietetic and nutritional invention.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

SH, MRS, NS and HH supervised the research process and provided critical feedback. YQO performed experiments, designed experiments, data analysis, wrote the manuscript and reviewed the manuscript. All authors read and approved the final version.

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