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Antioxidant activities and total phenolic content of immature and undersize Melon Manis Terengganu (*Cucumis melo* L.) peel, seed and flesh powder.

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Cucumis melo L. has high demand in certain areas of Malaysia for its best quality, however these fruits have become waste whereby certain fruits are discarded at immature or mature undersize stages. The present study was conducted to determine the antioxidant activities which consist of 2,2-diphenyl-1-picrylhydrazyl assay (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and Total Phenolic Content (TPC) of immature and undersize mature *Cucumis melo* L. powder peel, flesh and seed. The DPPH, ABTS and TPC content in the undersize mature peel powder was significantly higher than immature peel powder which was; TPC (4013.00 mg GAE/100g), DPPH (83.74% inhibition), and ABTS (63.13% inhibition). For the seed, the undersize mature seed powder showed higher TPC and antioxidant activities than immature seed powder where the TPC (1006.70 mg GAE/100g), DPPH (46.89 % inhibition), and ABTS (43.40% inhibition). Flesh of immature Melon Manis Terengganu powder had significantly higher TPC and antioxidant activities than undersize mature flesh powder where the immature flesh powder which the value of TPC was (1111.91 mg GAE/ 100g), DPPH (42.34%) and ABTS (36.20%). The study of immature and undersize peel, seed and flesh powder of *Cucumis melo* L. should be explored as these raw materials has the potential to be a good source of natural antioxidant compounds in future.

Keywords: Antioxidant, *Cucumis melo* L., mature, immature, peel, seed, flesh.

INTRODUCTION

Epidemiological studies now suggest more intake of antioxidant from fruits and vegetable sources due to its ability to reduce the occurrence of chronic diseases such as cardiovascular diseases, bowel disorders, and cancer (Navarro et al., 2011). Fruits and vegetables intake is highly recommended in daily diet because they have protective effects against diseases due to their presence of flavonoids, anthocyanins, and other phenolic compounds (Thakur, 2015). Human body naturally produces reactive oxygen species (ROS) such as superoxide anion radical, hydroxyl radical and hydrogen peroxide through enzymatic systems. This reactive oxygen species is beneficial to human body in small amounts however in larger quantities it can cause serious conditions like cancers, aging and others (Siddeeg et al., 2014). However, in Malaysia these fruits and vegetable residues are thrown when it does not meet up marketing quality which can possess a good nutritional characteristic. Melon Manis Terengganu (MMT) (*Cucumis melo* L.) is highly cultivated in countries like Africa and Asia (Nazeem et al., 2016). In Malaysia, Melon Manis Terengganu are discarded either immature or mature undersize stages from their tree to reduce nutritional

competition in order to produce the best quality fruit.

Cucumis melo L. has high economic value and it is easily cultivated all around the world due to its ability to adapt to different types of climate and soil (Wen et al., 2015). Melon is a fruit that is rich in folic acid, thiamine, riboflavin, pro-vitamin A and vitamin C (Vasundra Devi, 2011). The concentrations of sucrose, β-carotene, total sugars, soluble solids and 5-methyltetrahydrofolic acid are found in the different parts of fruits (Widowati, 2015). Melon has variety of aroma due to presence (Z.Z)-3,6-nonadien-1-ol and phenylethyl alcohol that imparts fresh and sweet- floral characters. According to Ismail et al. (2010), rock melon (*Cucumis melo* L.*)* has high antioxidant activity, ascorbic acid content and total phenolic content. Ong et al., (2019) reported that bioactive compounds in *Cucumis melo* L. provide a lot of benefits to human health. Besides, the phytochemicals compound found in Cucurbitacae family provides antioxidants and anti-inflammatory effect as reported by Ong et al., (2020a) and epigallocatechin gallate compound was reported to provide those effects (Ong et al., 2020b). However, there is lack of information regarding antioxidant activities content of Melon Manis Terengganu specifically in the seed, peel and flesh.

Thus, this paper reported the antioxidant activities which consist of DPPH, ABTS and TPC of different parts of Melon Manis Terengganu which in line with the scientist recommendations to obtain natural antioxidants that comes from plant by products instead of synthethic antioxidants which have been used in industries such as butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, dodecyl gallate, and tertiary butylhydroquinone (Navarro et al., 2016).

MATERIALS AND METHODS

METHODOLOGY

2,2-diphenyl-1-picrylhydrazyl assay (DPPH)

DPPH assay was measured as reported by Mallek-Ayadi et al., (2017) using stock solution of standard solutions of quercetin as 1 mg/ml in methanol. Different concentrations were used (7.82, 15.63, 31.25, 62.5, 125, 250 and 500 µg/ml in methanol) in 96 well microliter plate of 40 µL volume. DPPH solution that was prepared with 0.04 mg/ml concentration in methanol was added in volume of 160 µL in each well. DPPH and methanol were used as blank. The plate was placed in dark condition at 37°C for 30 minutes and gently shook. The absorbance was measured at 515 nm using microplate reader (Bio-tek Instruments, USA). Percentage DPPH scavenging activity was calculated as follows: [1-(absorbance of sample/ absorbance of blank)] x 100.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

ABTS was determined by procedure described by Navarro et al., (2016). ABTS was prepared in 7 mM concentrated solution using water. ABTS free radical was produced by the reaction of stock solution (3 ml) with 2.45 mM potassium persulfate (3 ml) with incubation at room temperature in dark condition. After 12-16h of incubation, ABTS solution was diluted in 80% ethanol to reach an absorbance of 0.7 at 734 nm. Ethanol (80%) without sample was used as blank control. The standard solutions were prepared with 80% ethanol with concentration of 7.82, 15.63, 31.25, 62.5, 125, 250 and 500 µg/ml. 200µL ABTS working solution and 10 µL of 80% ethanol was used as blank control. In standard curve wells, 10 µL of prepared Trolox standard solution and 10 µL of extract were added into sample wells. The plate well was then incubated for 5 minutes and absorbance was measured at 734 nm using microplate reader (Biotek Instrument, USA). The percentage ABTS scavenging activity for each sample triplicates were calculated as follows: [1- (abs of sample/ abs of blank)] x 100.

Total phenolic content (TPC)

Total phenolic content (TPC) of MMT peel, seed and flesh aqueous extracts was determined by FolinCiocalteu method as reported by Mallek-Ayadi et al., (2016). Gallic acid standard solution with concentration of (1.56-100) µg/ml was prepared with water. 50 µL of 1M sodium carbonate solution, 10% FolinCiocalteu (F-C) reagent, distilled water, extract (1 mg/ml) concentration was added to the 96-well plate. The blank used was distilled water. The 96 well-plate then was incubated for 60 minutes at room temperature in dark. Absorbance was measured at 750 nm with a Microplate Reader (Biotek, USA). Total phenolic content was expressed as µg Gallic Acid Equivalents (GAE) per mg dry plant material. Molarity (M) x Volume (L) x Molar mass = 1x0.001x106 = 0.106g/ 106mg of sodium carbonate.

RESULTS AND DISCUSSIONS

Table 1, Table 2 and Table 3 shows the value of antioxidant activities analyzed in immature and undersize mature peel, flesh and seed of Melon

Manis Terengganu powder. There was significant difference between all parts (peel, seed and flesh) of undersize mature in comparison with immature parts for all the TPC, TFC, DPPH, and ABTS at level $p<0.05$, except for ABTS of immature and undersize mature flesh.

Table 1: Total phenolic content and antioxidant activity (ABTS and DPPH) in peel of immature and undersize mature MMT *(Cucumis melo*L.)

Antioxidant Activities	Immature Peel	Undersized Mature Peel
TPC(mg GAE/100g)	1012.33±0.78[a]	4013.00 ± 1.24[b]
DPPH (%)	51.19± 0.94[a]	83.74 ± 1.40[b]
ABTS (%)	43.52± 0.36[a]	63.13 ± 0.40[b]

Values with different superscript letters within the same row are statistically different ($p<0.05$).

Table 2: Total phenolic content and anti-oxidant activity (ABTS and DPPH) in flesh of immature and undersize mature MMT *(Cucumis melo*L.).

Antioxidant Activities	Immature Flesh	Undersized Mature Flesh
TPC (mg GAE/100g)	1111.91 ± 0.95[b]	892.41 ± 0.80[a]
DPPH (%)	47.84 ± 0.31[a]	42.96± 0.35[a]
ABTS (%)	41.49 ± 0.23[a]	41.19± 0.01[a]

Values with different superscript letters within the same row are statistically different ($p<0.05$).

Table 3: Total phenolic content and anti-oxidant activity (ABTS and DPPH) in seed of immature and undersize mature MMT (*Cucumis melo* L.).

Antioxidant Activities	Immature Seed	Undersized Mature Seed
TPC(mg GAE/100g)	1071.31±3.00[a]	1006.70±3.00[a]
DPPH (%)	46.89 ± 0.35[a]	46.01 ± 0.03[a]
ABTS (%)	41.40± 0.50[a]	43.40 ± 0.39[a]

Values with different superscript letters within the same row are statistically different ($p<0.05$).

Total phenolic content (TPC)

Phenolic compound that is present in fruit peel is essential to act as a protector for inner flesh from insects and microorganisms. Apart from that, phenolic compounds also important in imparting the colour of the fruit (Jeong et al., 2014). Total phenolic content was significantly higher in the undersized mature (4013.00 ± 1.00) and immature (1012.33 ± 0.33) peel of the MMT.

In a similar study by Bidkar (2012), he concluded that MMT peel contains high amounts of phenolic compounds, which have been associated to protection against infections and environmental stresses. According to other study, the peel of the pumpkin contains the highest concentrations of soluble dietary fibre, phenolic compounds, flavonoids, and antioxidant activity. Liara et al. (2017) determined the total phenolic content of cucumber peels and pulps from various varieties. They discovered that when peels were compared to pulps, peels invariably contained a higher percentage of total phenolics. Mallek-Ayadi et., (2017) reported the same conclusion with two watermelon varieties.

Phenolic substances are secondary metabolites produced in fruits that are not uniformly distributed throughout the fruit tissues. Increased phenolic component concentrations in the peel's outer tissues are associated with the peel's primary natural function: pathogen and environmental stress resistance. As a result of this biological activity, phenolics display antiviral, antibacterial, and antitoxin effects. Biswas. (2006) on the other hand examined the phenolic compounds present in watermelon seeds, peel, pulp, and leaves and concluded that seeds and leaves had the most diverse array of phenolic compounds, while pulp contained the fewest. Numerous studies have also discovered a correlation between plant phenolic content and antioxidant activity, most likely as a result of their redox properties, which enable them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers.

2,2-Diphenyl-1-Picrylhydrazyl Assay (DPPH)

DPPH is free radical that is stable and remains undamaged by side reactions like enzymatic inhibition or metal ion chelation (Bahloul et al., 2009). This antioxidant potential in immature and mature *Cucumis melo* was studied using DPPH reagent in methanol solution. At absorption of 517 nm, DPPH solutions shows deep purple colour (Ismail et al., 2010). Total DPPH content was significantly higher in the undersized mature (83.74 ± 1.40) and immature (51.19 ± 0.94) peel of the MMT.

The antiradical activity of the MMT was investigated using the DPPH assay, which measured the reduction of the DPPH radical to

hydrazine as a result of their antiradical activity. These findings were consistent with previous research: in this experiment, the MMT peels extract was shown to be more active than the results published by Ismail et al., (2010). Seed extract had half the scavenging activity of that seen and reported by Ismail et al., (2010). The DPPH assay results imply that extracts can scavenge free radicals through electron or hydrogen-donating processes.

Furthermore, MMT DPPH activity was shown to be related to the amount of polyphenols, ortho-diphenols, flavonoids, and tannins, implying that radical scavenging activity of MMT peels is proportional to the amount of phenolic components. Synthetic antioxidants are widely utilized as additives in food, pharmaceuticals, and cosmetics today, although their safety has been questioned due to several components generated during their degradation in industrial processing, which may be hazardous or carcinogenic. As a result, using natural plant-based compounds to substitute manufactured molecules may be a viable option, not only because of their safety, but also because they protect food, feed, and derivatives from the harmful effects of natural oxidation. Synthetic antioxidants are known to scavenge free radicals, but due to their carcinogenic side effects, the hunt for effective and natural antioxidants has become critical. Natural antioxidants are thought to be more bioactive and safer. The major antioxidant activity of the sample in this investigation was measured using the DPPH test. A main antioxidant is a chemical that has the ability to eliminate or scavenge free radicals. The ability of the samples to scavenge free radicals is seen in the DPPH assay result as a color change from purple to yellow due to hydrogen donating ability. The principal antioxidant action is strong the faster the absorbance declines.

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

ABTS is likely similar to DPPH that both use strongly coloured stable radical compound but ABTS radical is generated by oxidizing it into radical cation while DPPH is already generated and commercially available (Morais et al., 2015). Total ABTS content was significantly higher in the undersized mature (63.13 ± 0.40) and immature (43.52 ± 0.36) peel of the MMT.

The reaction of the ABTS and radical cation generated in the assay with the antioxidant present in the sample is the basis of the ABTS assay. This method takes less time than the others and is also used to check the results obtained with DPPH because their antioxidant mechanisms are similar. The findings revealed that all sections of MMT have antioxidant activity, but to varying degrees. The results were identical to those of DPPH, indicating that the peel had the strongest antioxidant activity. The antioxidant activity varies in different areas of the body, which could be connected to the polarity of different solvents. It is possible to deduce from the results of a comparable experiment that the change in the polarity of the organic solvent. Another study found that the peels had higher levels of polyphenolic content and antioxidant activity than the pulps. Fruit peels, on the whole, have higher antioxidant activity than fruit pulp. Geographical location, cultivar type, harvest season, and storage conditions may all influence antioxidant activity.

CONCLUSION

Based on this analysis of antioxidant activities and total phenolic content, it can be concluded that Melon Manis Terengganu by products which consist such as peel, seed and flesh from immature or undersize fruit has the potential to be used as functional food ingredients or to be developed as functional food in future.

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CONFLICT OF INTEREST

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

AUTHOR CONTRIBUTIONS

MS performed the experiments and wrote the manuscript. NS, NH and ZZ supervised, design the experiment and reviewed the manuscript. All authors read and approved the final version.

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