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Antioxidant activity and total phenolic content in immature and mature rock melon (*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 undersized 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 undersized mature *Cucumis melo* L. powder peel, flesh and seed. The DPPH, ABTS and TPC content in the undersized mature peel powder was significantly higher than immature peel powder which was; TPC (4012.00 mg GAE/100g), DPPH (83.09% inhibition), and ABTS (64.27% inhibition). For the seed, the undersized mature seed powder showed higher TPC and antioxidant activities than immature seed powder where the TPC (1007.60 mg GAE/100g), DPPH (45.94 % inhibition), and ABTS (38.20% inhibition). Flesh of immature Melon Manis Terengganu powder had significantly higher TPC and antioxidant activities than undersized mature flesh powder where the immature flesh powder which the value of TPC was (1114.40 mg GAE/ 100g), DPPH (42.34%) and ABTS (36.20%). The study of mature peel, seed and immature 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 are 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 undersized 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 & 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 & 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. 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 in industries such as butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, dodecyl gallate, and tertiary butylhydroquinone (Navarro et al., 2016).

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 1mg/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 40µL volume. DPPH solution that was prepared with 0.04 mg/ml concentration in methanol was added in volume of 160µL in each well. Blank that was used in this assay was of DPPH and methanol. The plate was placed in dark at 37°C for 30 minutes and shaken gently. The absorbance was measured at 515nm 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 7mM concentrated solution using water. ABTS free radical was produced by reaction of stock solution (3ml) with 2.45mM potassium persulfate (3ml) with incubation at room temperature in dark. After 12-16h of incubation, ABTS solution was diluted in 80% ethanol to reach an absorbance of 0.7 at 734nm. 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 & 500µg/ml. 200µL ABTS working solution and 10µL of 80% ethanol was used as blank control. In standard curve wells, 10µL of prepared Trolox standard solution was added, and 10µL of extract into sample wells. The plate well was then incubated for 5minutes and abs was measured at 734nm using microplate reader (Biotek Instrument, USA). The percentage ABTS scavenging activity for each sample triplicates was 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 Folin Ciocalteu 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. 50µL of 1M sodium carbonate solution, 10% Folin Ciocalteu (F-C) reagent, distilled water, extract (1mg/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 750nm 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.

RESULT AND DISCUSSION

Table 1 shows the value of antioxidant activities analyzed in immature and undersize mature peel of Melon Manis Terengganu powder. There was significant difference between all parts (i.e. peel, seed and flesh) of undersized mature in comparison with immature parts for all the TPC, TFC, DPPH, & ABTS at level $p < 0.05$, except for ABTS of immature and undersized mature flesh.

Table 1. Total phenolic content and antioxidant activity (ABTS and DPPH) in peel of immature and undersized mature MMT *(Cucumis melo* L.). Significant different is determined by independent T-Test $p<0.05$ as compared as significant difference within row. The * denotes higher significance difference at level $p<0.05$.

Antioxidant Activities	Immature Peel	Undersized Mature Peel
TPC(mg GAE/100g)	1012.00 ± 0.78	4012.00* ± 1.24
DPPH (%)	50.33 ± 1.30	83.09* ± 0.74
ABTS IC50 (%)	43.25 ± 0.60	64.27* ± 0.98

Table 2. Total phenolic content and antioxidant activity (ABTS and DPPH) in flesh of immature and undersized mature MMT *(Cucumis melo* L.). Significant different is determined by independent T-Test $p<0.05$ as compared as significant difference in row. The * denotes higher significance difference at level $p<0.05$.

Antioxidant Activities	Immature Flesh	Undersized Mature Flesh
TPC(mg GAE/100g)	1114.40* ± 0.95	894.60 ± 0.80
DPPH (%)	48.23* ± 0.23	42.34 ± 0.50
ABTS (%)	4.50 ± 2.18	4.18 ± 1.54

Table 3. Total phenolic content and antioxidant activity (ABTS and DPPH) in seed of immature and undersized mature MMT *(Cucumis melo* L.). Significant different is determined by independent T-Test $p<0.05$ as compared as significant difference in row. The * denotes higher significance difference at level $p<0.05$.

Antioxidant Activities	Immature Seed	Undersized Mature Seed
TPC(mg GAE/100g)	976.20 ± 3.00	1007.60* ± 3.00
DPPH (%)	37.13 ± 0.57	45.94* ± 0.42
ABTS (%)	31.21 ± 0.28	38.20* ± 0.38

Total phenolic content (TPC)

Phenolic compounds 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 and appearance of the fruit (Jeong et al., 2014). Using the Folin-Ciocalteu method, the total phenolic content of mature Melon Manis Terengganu peel was 4012.00 mg GAE/100gm higher than the total phenolic content of immature peel (1012.00mg GAE/100g) (table 1). The polyphenol content in mature Melon Manis Terengganu peel was higher than total polyphenols in watermelon peel (335.3mg/100g extract) found by Duda-Chodak&Tarko (2007) and cantaloupe peels (470mg/100g extract) (Ismail et al. 2010). Phenolic phytochemicals possess important nutritional, organoleptic and antioxidant properties in foods. Thus, mature peel has good potential to be utilized as polyphenol compounds in food industry. Phenolic compound has bioactive potential that is of the main class of secondary metabolites (Ahmad et al., 2011). The total phenolic content in mature flesh (1114.40 mg GAE/100g) was significantly higher than immature flesh (894.60 mg GAE/100g). However, the total phenolic content for both immature and mature flesh is lower than Cultivar

honey dew (1630mg GAE/100g) and Cultivar Yellow type V Reticulatus (1790mg GAE/100g). Phenolic compounds are also known as free radical terminators. Scientist are now more considering natural antioxidants that comes from plants to be used in food industry rather than synthethic ones. Exploration of natural phenolic compounds from Melon Manis Terengganu can be an alternative to replace synthethic antioxidant and maintain food from oxidative deterioration (Ahmad et al., 2011). Antioxidants delays oxidation of substrate, and phenolic compounds are known to show these properties in fruits. Furthermore, polyphenols are associated with reduction in lipid oxidation, DNA mutation, tissue damage, and protein cross-linking (Ahmad et al., 2011). The total phenolic content of mature seed is 1007.60mg GAE/100g (Table 2). This amount was higher than cantaloupe seeds (285 mg/100g) and Maazoun cultivar seeds (304.10mg/ 100g) (Zeb, 2016). Due to diverse chemical structures of phenolic compounds, phenolic phytochemicals have the tendency to converse cellular components in human body against damage causing free radicals (Bahloul et al., 2014). From this study, the mature Melon Manis Terengganu seed has the highest phenolic content than mature peel and flesh, also higher than immature peel, seed and flesh. Polyphenols has the ability to prevent chronic diseases such as cardiovascular and cancer diseases (Liara et al., 2013).

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 chelations (Bahloul et al., 2009). This antioxidant potential in immature and mature *Cucumis melo* was studied using DPPH reagent in methanol solution. At absorption of 517nm, DPPH solutions shows deep purple colour (Ismail et al., 2010). The antioxidant potential in immature flesh (48.23 mg QE/ml) was higher than mature flesh (42.34 mg QE/ml). The scavenging activity of cantaloupe was lower than Melon Manis Terengganu flesh at only 3.33 mg QE/ml) and watermelon at (6.29 mg QE/ml) (Zeb, 2016). The deep purple colour was faded because the free radical was quenched by antioxidants that is present in Melon Manis Terengganu. DPPH assay is mostly used because it is simple, stable and requires short time for analysis (Ahmad et al., 2011). The DPPH radical scavenging activity of mature peel was significantly higher than immature peel (83.09% and 50.33%) respectively. According to Ayden & Gocmen (2015), pumpkin peel only had 18% radical scavenging activity and watermelon with 38.89% which was much lower than both immature and mature peel of melon manis Terengganu. Arora (2011) stated that melons contain antioxidant properties due to presence of certain compounds like carotenoids, lycopene and glucose oxidase enzyme. These phenolic compounds are often categorized as hydrophilic antioxidant and are responsible for antioxidant properties in plants (Liara et al., 2013). The mature seed (45.94%) contained significantly higher radical scavenging activity than immature seeds (37.13%). The DPPH radical scavenging found in pumpkin seed was 23% and cantaloupe seed was 20% (Ayden &Gocmen., 2015) which was lower than of immature and mature MMT seeds. From this analysis, the antioxidant properties in mature parts were significantly higher than the immature parts of MMT fruit, this may be due to certain compounds like gallic acid and quercetin acid low in immature and may increase with time reaching its highest value once the fruit starts ripening. (Zeb, 2016)

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). In this analysis, the mature peel (64.27%) contained significantly higher antioxidant properties than immature peel (43.25%). In case of maazoun cultivar melon the free radical scavenging activity of the peel was (26%) (Mallek-Ayadi et al., 2016) only which was much lower than Melon Manis Terengganu. ABTS assay has advantage over antioxidant systems that is freely soluble in both organic and aqueous

solvent and can screen both hydrophilic and lipophilic compounds (Wen et al., 2015). Based on the result achieved for seed of mature and immature MMT, the mature seed contained higher ABTS free radical scavenging activity which was 38.20%, however it was lower than the ABTS radical scavenging activity found in pumpkin seeds (41%) and cantaloupe seeds (42.36%) (Mallek-Ayadi et al., 2017). The immature flesh contained significantly higher ABTS free radical scavenging activity as compared with mature flesh which was at 39.11% and 36.20% respectively. However, both these mature and immature flesh had higher ABTS radical scavenging activity than those reported in pumpkin flesh (10%) and cantaloupe seed (21.20%) by Al-Sayed & Ahmed (2013). Based on this result, it can be stated that the phenolic and compounds that is present in fruits like melon has the tendency to protect human against free radical induced damage with their respective antioxidant effect due to their diverse chemical structures (Bahloul et al., 2014).

CONCLUSION

Based on this analysis on antioxidant activity and total phenolic content showed higher value than most of reported fruits and vegetables of Cucurbitacae family. Melon Manis Terengganu are regularly grown and consumed in tropical countries and this report suggest that this fruit has potential to be developed into functional food.

CONFLICT OF INTEREST

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

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AUTHOR CONTRIBUTIONS

MS perform electronic database search 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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