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β-carotene and Antioxidant Analysis of three Different Rockmelon (*Cucumis melo* L.) Cultivars

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Abstract: Rockmelon is an important commercialized fruit that belongs to one of Cucurbitaceae family. In this experiment, three rockmelon cultivars *viz* Honeymoon, Champion and Glamour were selected for β-carotene, Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and radical scavenging activity (SC_{50}) analysis. Honeymoon cultivar gave the highest percentage for β-carotene content (9.7×10^{-4}%). The aqueous extract of each hybrid fruit's skin, flesh and seed was used in determining TPC, TFC and SC_{50}. It was discovered that Glamour fruit's skin had the largest amount of total phenolic content (52.5 mg Gallic Acid Equivalent (GAE) g^{-1}) and Champion seed gave the highest value for flavonoid content (861 mg Catechin Equivalents (CE) g^{-1}). For DPPH test, Glamour seed performed the highest radical scavenging activity at the lowest concentration (250 μg mL^{-1}). In conclusion, Glamour seed, Honeymoon and Glamour fruit flesh gave better antioxidant activities compared to other extracts. Thus, these results suggested that aqueous extract of *Cucumis melo* L. cultivars may serve as a potential source of natural antioxidant for food and nutraceutical application.

Key words: Rockmelon, cultivars, total phenolic content, total flavonoid content, DPPH, β-carotene content

INTRODUCTION

Rockmelon is fruit from Cucurbitaceae family that related to pumpkin, squash, cantaloupe and other plants that vines on the ground. It habitat was found in temperate region of Africa, Central Asia, and Mediterranean. Ritschel *et al.* (2004) and Ismail *et al.* (2010) stated that the Cucurbitaceae family includes with several species of cultivated plants with great economic importance, including watermelon (*Citrullus lanatus* L.), squash (*Cucurbita maxima* L.), cucumber (*Cucumis sativus* L.) and cantaloupe (*Cucumis melo* L.). This fruit could contribute to the country economically including the wild types and numerous varieties (Silberstein *et al.*, 1999). In Asia and Africa it is commonly consume as the selective food and fruit. Moreover, Levi *et al.* (2001) also discovered that almost 2% of the world plant production was dominated by rockmelon. The fruit contained with sweet, juicy tastes, pleasant flavour and high nutritional value. Almost hundred of rockmelon being cultivated and the varieties fall into three broad classes that based on how the seed were developed which are open-pollinated, F_1 hybrid, and triploid or seedless (Buchanan *et al.*, 2000). The hybrid plant was specifically defined as a selective breeding product after undergo cultivation process and was in large demand by many of planters. It consists of many interested gene that allow them to further propagate actively.

Cantaloupes with diverse group of fruits includes with orange flesh color cantaloupes, green flesh honeydew, and mixed melons. Rockmelon cultivar with mostly orange color fruit flesh believed to have high quantity of β-carotene. β-carotene contributes to orange color for the fruits and act as antioxidant where it was used to protect the body from free radicals. β-carotene in diet may lower the risk of heart disease and cancer (Gabriele *et al.*, 2000; Bjelakovic *et al.*, 2007). It also provided with vitamin A that important for the body that contribute for good eye vision, eye health (Herrick *et al.*, 2000) and strong immune system (Sluijs *et al.*, 2009).

Howard *et al.* (2003) and Islam *et al.* (2003) had found out that genetic factors could play important role in the formation of secondary metabolites including phenolic acids. Thus, in this research different rockmelon cultivars were used. Different parts of fruit gave different result for antioxidant properties. Previous study by Vouldoukis *et al.* (2004) had showed that cantaloupe pulp extract possesses high antioxidant and anti-inflammatory properties. On the other hand, Ismail *et al.* (2010) found that methanolic extracts of cantaloupe leaf and stem parts

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exhibited good antioxidant activities. Antioxidant assessment on different parts of *C. melo* cultivars are very limited (Mariod and Matthaus, 2008). Thus, the aim of this research was to determine the β-carotene, phenolic, flavonoid content and antioxidant activities of aqueous extracts from different parts of different *C. melo* cultivars.

MATERIALS AND METHODS

Sample preparation: Rockmelon of different cultivars were grown under rain shelter at MARDI, Serdang by applying fertigation system for irrigation. At 42 to 46 days after pollination, the fruits achieved it maturation stage and ready to be harvested (Fig. 1). At this stage, the pH value and the Brix percentage of the rockmelon cultivars were measured at the range of 4.9-5.6 and 11-17%, respectively.

Sample extraction: The extraction was carried out according to Wong *et al.* (2006) with some modification. Twenty gram of each rockmelon cultivar fruit part (seed, fruit flesh and skin) were ground and dissolved in 100 mL of distilled water into fine slurry. The fruit parts extractions were heated and continuously stirring at 60°C for 3 h. Then, the extracts were filtered and concentrated using a freeze dried instrument. The water extracts were kept in refrigerator at -4°C. The freeze dried aqueous extracts that obtained were used directly for total phenolic and flavonoid contents determination and also for the assessment of antioxidant capacity through radical scavenging determination by using DPPH method.

Total phenolic assay: Total phenolic was determined according to Kumaran and Karunakaran (2007) method with some modification. The total phenolic content of plant extracts was determined using Folin-Ciocalteu's Reagent (FCR) and calculated using gallic acid calibration curve as a standard. Stock solution of gallic acid was prepared at 0.1 mg mL^{-1}. About 0.1 mL of diluted extract or standard solution (0, 0.5, 1.0, 2.0, 3.0 and 5.0 μg mL^{-1}) was mixed with 1 mL of 50% Folin-Ciocalteu's reagent and shaken. After 2 min, 2 mL of 20% sodium carbonate,

Fig. 1(a-c): (a) Rockmelon of each cultivars being planted through fertigation technique (b) Image of ripe rockmelon fruit and the skin was netted form (c) Rockmelon fruit cut in halves shown orange color flesh structure

(Na_2CO_3) was added to the mixture. The mixture was shaken thoroughly and made up to 10 mL using distilled water. The mixture was allowed to stand for 2 h in the dark. The absorbance at 750 nm was determined using UV-Vis spectrophotometer. The absorbance of extracts was compared to gallic acid calibration curves. The total phenolic content of each extract was expressed on fresh weight basis as mg g^{-1} Gallic Acid Equivalents (GAE). All determinations were carried out in triplicates.

Total flavonoid assay: Total flavonoid was measured with an aluminum chloride colorimetric assay whereby catechin was used as a standard solution developed by Zhishen *et al.* (1999) with some modification. Stock solution of catechin was prepared at 1000 μg mL^{-1}. A 1 mL aliquot of appropriately diluted standard solution of catechin (0, 200, 400, 600, 800 and 1000 μg mL^{-1}) or diluted extract was added to a 10 mL volumetric flask containing 4 mL distilled water. At zero time, 0.3 mL 5% $NaNO_2$ was added to the flask. After 5 min, 0.3 mL 10% $AlCl_3$ was added. At 6th min, 2 mL 1 M NaOH was added to the mixture. Immediately, the reaction flask was diluted to volume with the addition of 2.4 mL of distilled water and thoroughly mixed. Absorbance of the pink color mixture was determined at 510 nm using UV-Vis spectrophotometer. Total flavonoid of extract was expressed on fresh weight basis as mg g^{-1} Catechin Equivalents (CE). Samples were analyzed in three replications.

Radical scavenging activity by DPPH: DPPH assay with some modification was performed according to Tagashira *et al.* (1995). Stock solution of crude extracts or standard solution of quercetin was prepared as 1 mg mL^{-1} in methanol. The solutions were diluted to different concentrations (7.82, 15.63, 31.25, 62.5, 125, 250 and 500 μg mL^{-1} in methanol) in a 96-well microliter plate. Then, 5 μL of DPPH solution (prepared as 2.5 mg mL^{-1} in methanol) was added to each well. A blank solution that served as control was prepared containing the same amount of methanol and DPPH. The plate was shaken gently and placed in the dark for 30 min at 37°C. The absorbance was measured at 515 nm using microplate reader. The experiment was performed in triplicates and the graph was plotted with the means values. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{Redical scavenging activity } (\%) = I - \frac{A_{sample}}{A_{control}} \times 100$$

where, by A_{sample} and $A_{control}$ are absorbance of sample and control. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity.

β-carotene analysis: β-carotene content was analyzed according to method done by Cyanotech Corporation (2002). Five milliliter of fruit juice for every rockmelon cultivars were used and filled into a boiling tube. Firstly vortex test tubes were placed in the water bath for 30 min and at every 10 min, the tubes were taken out and continuously vortex.

Five milliliter of methanol were added into each test tubes, vortex and centrifuged at 4200 rpm for 3 min. The supernatant were taken out and moved into a new flask. Steps were repeated until all the supernatant is collected and the flask was filled up with methanol until volume reached 25 mL (methanol extract).

Eight milliliter of the prepared methanol extract, 5 mL of heptane and 1.5 mL of saturated potassium hydroxide were moved into a new test tube and left for saponification for 15 min and lightly vortex. The tubes were centrifuged at 4200 rpm for 3 min. Pipette was used to remove the heptane layer (upper layer) and put into a new 10 mL volumetric flask. Approximately 1 mL of fresh heptane was gently added to the tube and washed the interphase. The upper layer was added into the 10 mL volumetric flask. Three milliliter of fresh heptane was added to the tube. The tube was capped and inverted 8 times to allow any remaining beta-carotene in the methanol to enter the heptanes and separate from the methanol. The upper layer was pipette into the 10 mL volumetric flask. The 10 mL volumetric flask up volume was brought up with fresh heptane. The volumetric flask was capped and inverted to mix up the volume. Approximately 5 mL of the heptane extract was removed into a new centrifuge tube and an equal amount of distilled water was added and vortex vigorously for 5 sec. The tubes were centrifuged for 3 min at 4200 rpm. On the spectrophotometer, the absorbance was read at 436 nm of the extract against a heptane blank. The β-carotene percentage was calculated by using the formula:

$$\text{β-carotene } (\%) = \frac{\text{Abs } 436}{196 \times (\text{wt. (mg)} \times \text{dry wt.})} \times 25 \text{ mL} \times 1.25 \times 100 \times 0.84$$

Statistical analysis: The experiments were replicates three times and data obtained were analyzed through one way ANOVA on the 0.5 probability level by using the SAS software.

RESULTS AND DISCUSSION

Total phenolic content: Total phenolic content of aqueous extracts F_1 hybrid rockmelon different part (seed, fruit' skin and flesh) were determined by extrapolation of gallic acid calibration curve ($y = 0.086x-0.010$, $R^2 = 0.986$) and expressed in milligrams of gallic acid (mg GAE g^{-1}) (Fig. 2).

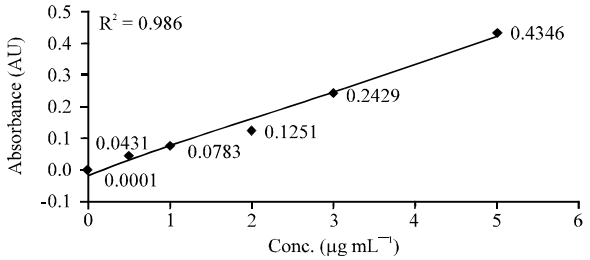

Fig. 2: Gallic acid calibration curve of absorbance vs. concentration

Table 1: Total phenolic content of samples

Samples	Phenols (mg GAE g^{-1})
Skin	
Champion	46.8±2.8
Glamour	52.5±2.9
Honeymoon	45.7±0.5
Seed	
Champion	40.7±0.7
Glamour	44.9±5.4
Honeymoon	41.7±0.9
Flesh	
Champion	33.8±6.0
Glamour	47.4±5.4
Honeymoon	28.4±0.7

Values are Average±SD

The Folin-Ciocalteu's assay was used to determine the total phenolic content in the sample. Reaction of phenolic compounds with FCR must under basic condition. Thus, sodium carbonate aqueous was added to the mixture to make the reaction occur. According to Roginsky and Lissi (2005) and Huang *et al.* (2005), the intensity of blue coloration produced is proportional to the total quantity of phenolic compounds present in the testing samples.

Table 1 presents the total phenolic content of rockmelon cultivars aqueous extract. The total phenolic content of rockmelon cultivars was varied from 52.5 and 28.4 mg GAE g^{-1} extract. Glamour fruit's skin and flesh had the highest amount of total phenolic content (52.5 mg GAE g^{-1}) whereas the lowest content was measured in the Honeymoon fruit's flesh (28.4 mg GAE g^{-1}) ($p<0.05$). Total phenolic content of rockmelon cultivars extract was arranged in the following descending order: Glamour fruit's skin>Glamour fruit's flesh>Champion fruit's skin>Honeymoon fruit's skin> Glamour seed>Champion seed>Honeymoon seed> Champion fruit's flesh>Honeymoon fruit's flesh.

Observation has proved that Glamour fruit's skin and flesh had the most intensity of blue coloration whereas Honeymoon fruit's flesh had the lowest intensity of blue coloration. The highest phenolic contents in the rockmelon cultivar may be due to high phenolic compounds contain in the extract such as flavanoids, triterpenoids, phenols and tannins.

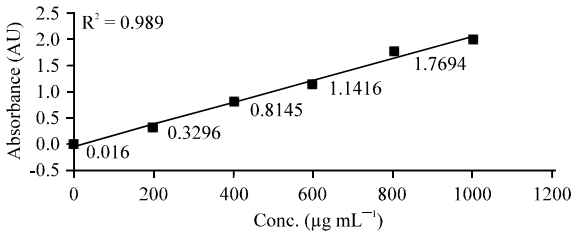

Fig. 3: Catechin calibration curve of absorbance vs. concentration

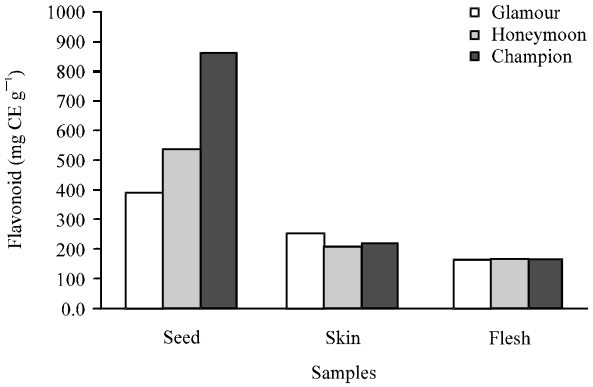

Fig. 4: Total flavonoid content (mg CE g^{-1}) of three rock melon cultivar at different parts (seed, fruit's skin and flesh), Values were expressed in Means±SD (n = 3)

Indeed, results shown that Glamour cultivar had the most phenolic content compared to the other varieties especially at it fruit's skin. Chang *et al.* (2001) had stated that the antioxidant activities in the plant are associated with total phenolic content. This may be due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers.

Total flavonoid content: Total flavonoid content of aqueous extracts of different parts for each cultivars (seed, fruit skin and flesh) were determined by extrapolation of catechin calibration curve ($y = 0.002x-0.027$, $R^2 = 0.989$) and expressed in milligrams of Catechin (CE)/gram (Fig. 3). In this study, aluminum chloride colorimetric was used and it is an assay specificity to explore typical structure of flavonoids. The total flavonoid content of rockmelon cultivars was varied from 861.0 and 161.0 mg CE g^{-1} extract. Champion seed had the highest amount of total flavonoid content (861.0 mg CE g^{-1}) and the lowest was Glamour fruit's flesh ($p<0.05$). From the results shown in Fig. 4, total flavonoid content of rockmelon cultivars extracts was arranged in the following sequence: Champion seed>Honeymoon seed>Glamour seed>Glamour fruit's skin>Champion

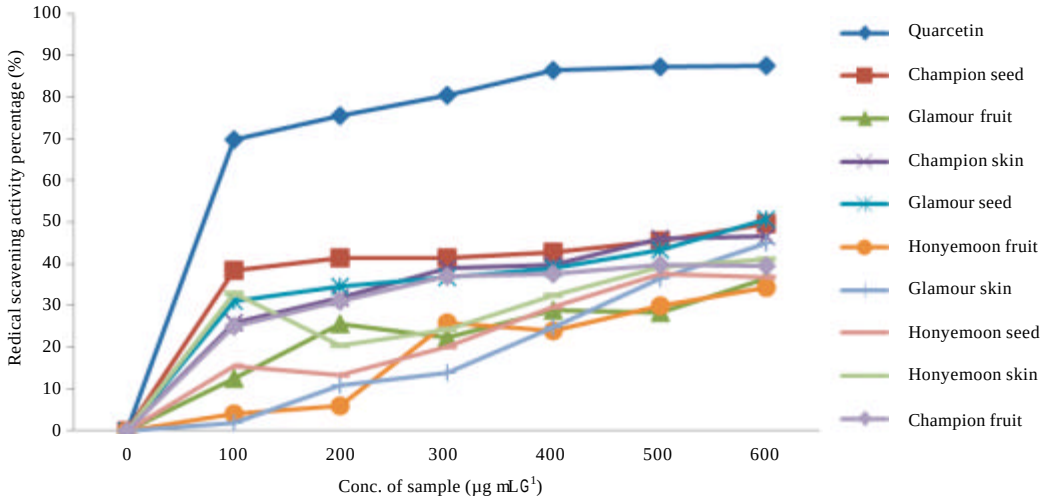

Fig. 5: Radical scavenging activities of samples with different concentration, Values are Mean±SD (n = 3)

Table 2: Flesh color and β-carotene percentage of rockmelon cultivars

Rockmelon cultivar	Flesh color	β-carotene (%)
Glamour	Slightly orange	5.2×10^{-5}
Champion	Most orange	3.4×10^{-4}
Honeymoon	Whitish orange	9.5×10^{-4}

fruit's skin>Honeymoon fruit's skin>Honeymoon fruit's flesh>Champion fruit's flesh>Glamour fruit's flesh ($p<0.05$).

Yanishlieva-Maslarova (2001) had mentioned that flavonoids were very effective antioxidants which it was commonly and widely distributed group of plant phenolic compound. From the result, seed of each rockmelon cultivars showed the most flavonoid content compared to the other part especially for the Champion cultivar.

Radical scavenging activity: The results obtained from the aqueous extracts of rockmelon cultivars were compared with quercetin as standard reference. The reduction in DPPH radical was determined by the decrease of its absorbance at 515 nm (in methanol) induced by antioxidants. In the present study, the radical scavenging activity for the aqueous extracts of rockmelon cultivars at different parts (skin, seed and flesh) were estimated using DPPH radical assay. DPPH method was quick, reliable and reproducible method to search the *in vitro* general antioxidant of pure compounds as well as plant extracts (Koleva *et al*., 2002). This method depends on the reduction of the purple DPPH by accepting electron or hydrogen radical from antioxidant (A-H) to a stable diamagnetic molecule (yellow-coloured diphenyl picrylhydrazine).

$$\underset{\text{(purple colour)}}{DPPH^{\bullet}} + A\text{-}H \rightarrow \underset{\text{(yellow colour)}}{DPPH\text{-}H} + A^{\bullet}$$

The degree of discoloration indicated the scavenging potential of the antioxidant compounds or extracts in the term of hydrogen donating ability (Mosquera *et al*., 2007).

Figure 5 shows all extracts and standard have increase in activity with increases of concentration (7.82-500 µg mL^{-1}). Based on the figure, Glamour seed had decreased the DPPH solution absorbance by half (SC_{50}) at smallest concentration (250 µg mL^{-1}) followed by Champion seed at SC_{50} = 270 µg mL^{-1}, Glamour fruit skin at SC_{50} = 320 µg mL^{-1}, Champion fruit skin at SC_{50} = 390 µg mL^{-1}, Honeymoon seed at SC_{50} = 450 µg mL^{-1} and Honeymoon fruit skin at SC_{50} = 500 µg mL^{-1}. Study shows that Glamour fruit skin had the highest antioxidant activity at 500 µg mL^{-1} followed by Glamour seed, Champion seed, Champion fruit skin, Honeymoon seed, and fruit skin. However, radical scavenging activity of Glamour and Honeymoon fruit flesh had decrease the DPPH solution absorbance less than 50% at 500 µg mL^{-1}. There are former statements on the contribution of phenolic compound in antiradical activity of cantaloupe extracts. Indicating that, DPPH radical scavenging activity rockmelon cultivar extracts was highly related to the amount of phenolic compounds present in the extracts. This was proved by the highest value of phenolic content in Glamour cultivar and followed with highest result of antioxidant activity.

β-carotene content: Result shows the percentage of beta carotene in the rockmelon cultivars with its flesh color (Table 2). Honeymoon with whitish orange fruit's flesh

color had the highest values for β-carotene (9.5×10^{-4}%) ($p<0.05$). This then followed by most orange color and slightly orange color fruit respectively. The result contrast with Teow *et al*. (2007) finding that the orange fleshed color fruit or vegetable had the highest amount of β-carotene.

The low orange color intensity for Honeymoon cultivar was able to give the highest value of β-carotene compared to Champion cultivar with mostly orange color. This different quantity on formation of secondary metabolites (beta carotene) between all fruit cultivars may influenced by genetic factors and plant growing condition (Howard *et al*., 2003; Islam *et al*., 2003).

From the result, Champion with high intensity orange color had shown high total phenolic and flavonoid content. This finding had supported research done by Teow *et al*. (2007) that orange flesh of clone plants had shown high antioxidant activities and also contained substantial amounts of phenolic compounds.

CONCLUSION

This study provided evidence that Honeymoon cultivar contained the highest value for β- carotene with small different to Champion fruit. Then, it also proved that all studied aqueous extracts of rockmelon cutivars at all parts (seed, fruit's skin and flesh) contained phenolic and flavonoid compounds. Moreover, DPPH radical scavenging activity of three varieties of the rockmelon cultivar's seed, fruit skin and flesh extract had radical scavenging activity in decreasing order: Glamour fruit's skin>Glamour seed>Champion seed>Champion fruit's skin>Honeymoon seed>Honeymoon fruit's skin> Glamour fruit's flesh>Champion fruit's flesh> Honeymoon fruit's flesh. Besides, present study also showed that Champion, Honeymoon and Glamour skins, flesh and seeds showed radical scavenging activity more than 50%. Ultimately, all skin, seed and fruit of all rockmelon cultivars are good sources of natural antioxidant. Overall, Glamour has better antioxidant activity compared to other extracts.

In addition, study also shows these plant varieties have high pontential to scavenge free radical molecules to protect cells from oxidative stress in human body. Therefore, these plant cultivars can reduce the rate of diseases among public. From this experiment, all three rockmelon cultivars were discovered contained wide nature antioxidants or antiradical that help to heal plenty of diseases. There have lots of compounds that have not been studied yet among these varieties of plant. For a recommendation, it is good to isolate and identified the compounds related to this antioxidant activity. Hence, further study can be done to identify which compounds contribute to the antioxidant activity. In addition, several antioxidant assays such as FRAP, FTC and TBA assay could be used to determine the antioxidant activity of each hybrid rockmelon. Therefore, comparative assay can be done to strengthen the results obtained.

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