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Effect of drying temperatures and extraction solvents on total phenolic, flavonoid contents and antioxidant properties of immature Manis Terengganu Melon (*Cucumis melo*)

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ABSTRACT

The aim of this study was to determine the effects of drying temperature (40, 50 and 60 °C) on the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity on immature Manis Terengganu Melon. The extraction was performed using different solvent (water, methanol and ethanol). The TPC, TFC and antioxidant activity were determined by using Folin-Ciocalteu method, Aluminium Chloride method, (2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and Free Reducing Antioxidant Power (FRAP assay), respectively. Drying temperature at 40°C showed the highest value of TPC extract. However, drying temperature at 50 and 60 °C showed the higher DPPH and FRAP values, respectively. Comparison between extraction solvents, methanol is the most efficient solvent to produce the highest value of TFC. Meanwhile, the used of water extract resulted in higher TPC and FRAP value compared to ethanol and methanol. The present obtained result may be used in food industries for developing of functional food ingredient.

Keywords: Manis Terengganu Melon, *Cucumis melo*, extraction, phenolic, flavonoid, antioxidant

ABSTRAK

Tujuan kajian ini adalah untuk mengkaji kesan suhu pengeringan (40, 50 and 60 °C) terhadap jumlah kandungan fenol (TPC), flavonoid (TFC) dan aktiviti antioksidan pada Manis Terengganu Melon yang belum matang. Semua sampel diekstrak dengan menggunakan pelarut yang berbeza (air, metanol dan etanol) sebelum dianalisis. TPC, TFC dan aktiviti antioksidan ditentukan dengan menggunakan kaedah Folin-Ciocalteu, kaedah klorida aluminium, kaedah 2,2-difenil-1-picrylhydazyl (DPPH) dan asai penurun Ferik (FRAP). Pengeringan pada suhu 40 °C menunjukkan jumlah TPC dan TFC tertinggi. Namun, suhu pengeringan pada 50 dan 60 °C menunjukkan nilai DPPH dan FRAP yang lebih tinggi. Dengan membandingkan pelarut pengekstrakan, metanol adalah pelarut yang paling efisien dengan nilai TFC, DPPH dan FRAP tertinggi. Walau bagaimanapun, pengekstrakan menggunakan air menghasilkan jumlah TPC yang lebih tinggi berbanding dengan etanol dan metanol. Hasil kajian ini mungkin boleh digunakan dalam industri makanan untuk membangunkan bahan makanan berfungsi.

Kata Kunci: Manis Terengganu Melon, *Cucumis* melo, ekstrak, fenol, flavonoid, antioksida

INTRODUCTION

Manis Terengganu melon is one of the rock melon species under Cantaloupe family that originated from Terengganu, Malaysia. It has a smooth yellow-golden without netted beige of skin and together with orange-coloured flesh just likes other rock melon. In order to get high quality of Manis Terengganu melon, from six melons for each tendril, only one being left and keep grow until matured to reduce competition and get full nutrition. The immature Manis Terengganu Melon which have a light green colour skin and flesh with 4 to 5 °Brix would be removed and considered as a waste.

Phenolic compounds have a positive effect on human health by defending the body against free radicals damage due to its strong antioxidant potency. Previous study reported that cantaloupe extract possesses high antioxidant (Ismail et al., 2010). Polyphenols is example of antioxidant compound where antioxidant activity can aid in reducing the risk of certain diseases related to free radical. Flavonoids are part of a group of phenolic acids that could be found naturally in fruit and possesses a series of biological properties which acting on biological systems as antioxidants. Rolim et al. (2018) reported the presence of flavonoid contribute to antitumor biological activity of melon.

Preservation by using suitable drying temperature with suitable solvent extraction can help in extending the shelf life and retaining the antioxidant respectively. However, the improper selection of drying temperature will exposed the sample to thermal degradation. Thus, the degraded product is low nutritional value and consequently, hampers the process of producing high quality products (Muhamad et al., 2015).

Successful extraction of phenolic compounds and antioxidants is essentially influence by type of solvent used. However, Thoo et al. (2013) reported that there is no general solvent that can accomplish an extraction of all phenolic compounds from the plant material. Therefore this study was carried out to determine the effect of drying temperature and different extraction solvents (water, methanol and ethanol) on total phenolic contents, total flavonoid content and antioxidant properties of immature Manis Terengganu melon.

MATERIAL AND METHODS

Chemicals and Plant Material

Methanol, ethanol, Follin reagent, sodium hydroxide, sodium nitrate, sodium acetate, aluminum chloride, acetic acid, 2,4,6-tripyridyl-s-triazine, hydrochloric acid, ferric chloride and HPLC grade acetonitrile were purchased from Merck (Darmstadt, Germany). Gallic acid and quercetin standards were from Acros Organics (NJ, USA). DPPH and sodium carbonate were obtained from Sigma–Aldrich (St. Louis, MO, USA).

The fresh immature MT 1 with the 4° Brix to 5° Brix were collected at Alor Lintang, Besut, Terengganu.

Preparation of Samples

The Manis Terengganu melon were washed under tap water to remove any dirt, dust or foreign matters. The fruits skin were peeled and the flesh were sliced and soaked in 0.2% sodium metabisulphite for 15 min. Then, it were dried in lab dryer at 40 °C, 50 °C and 60 °C until a constant weight were obtained. The sample powder were formed by grinding the dried immature MT 1 using stainless steel grinder and kept in the airtight container at room temperature prior to extraction.

Extraction Process

Water, ethanol and methanol were used in the extraction process. Sample extraction were performed described by (Yee, et al., 2012) with some modification. Sample powder was mixed with solvents at the ratio of 1:10 (w/v) and were shaken at 150 rpm at ambient temperature for 2 hours by using orbital shaker. The supernatants was collected by centrifugation at 1800 g for 15 min and filtered through Whatman filter paper No. 1 to obtain a clear solution.

Analysis

Total Phenolic Content (TPC)

TPC of sample was analysed using Folin-Ciocalteau assay described by Muhamad et al. (2014). 0.4 ml sample of the extracts was mixed with 2 ml of 10-fold diluted Folin-Ciocalteau reagent. After 5 min, 1.6 ml of 7.5% (w/v) sodium carbonate was added. The solution would be vortex mixed and allowed to stand at room temperature for 60 min. Then, the absorbance was measured at □ = 765 nm using a calibrated UV-visible spectrophotometer (Shimadzu UV-1601PC, Japan) against a blank of methanol. The result was expressed as mg gallic acid equivalent per hundred grams of sample through the calibration curve with gallic acid.

Total Flavanoid Content (TFC)

TFC of sample was determined by using method described by Muhamad et al. (2014). 1 ml of sample extracts was diluted with 4 ml distilled water and added with 0.3 ml 5% (w/v) $NaNO_2$. After 5 min, 0.3 ml of (10% w/v) $AlCl_3$ was added. After 6 min, 2 ml of 1 M NaOH was added and the volume made up to 10 ml immediately by the addition of 2.4 ml distilled water. The solution was mixed vigorously and the absorbance of the solution was measured using a calibrated UV-visible spectrophotometer (Shimadzu UV-1601PC, Japan) against a blank of methanol at □ = 510 nm. The result was expressed as milligrams of quercetin equivalents per hundred grams of sample by comparison with the quercetin standard curve.

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

The free-radical scavenging (antioxidant) activity of the sample extract was measured by means of DPPH assay according to Norrizah et al. (2012). Stock solution of extracts or standard solution of quercetin was prepared as 1 mg/mL in methanol. The solutions were diluted to different concentrations (7.82, 15.63, 31.25, 62.5, 125, 250 and 500 μg/mL in methanol) and 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 □ = 515 nm using microplate reader (Bio-Tek Instruments, USA). Triplicate measurements was carried out and the percentage of DPPH scavenging activity was calculated as follows:

[1 - (absorbance of sample/absorbance of blank)] x 100.

Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant capacity of MT 1 extracts were determined using FRAP assay described by Alam et al. (2013) with some modification. Acetate buffer (300 mM) was prepared using 3.1 g sodium acetate, 16 ml glacial acetic acid and 1000 ml distilled water. Dry 2,4,6-tripyridyl-s-triazine (0.031 g) was weighed and dissolved in distilled water in order to form 10 mM 2,4,6-tripyridyl-s-triazine. The working FRAP reagent was prepared by mixing 10 ml of acetate buffer, pH 3.6 with 1 ml of 2,4,6-tripyridyls-triazine in 40 mM hydrochloric acid and 1 ml of 20 mM ferric chloride. An aliquot of 8.5 □l of extract would be added to 275 □l of diluted FRAP reagent using a microplate and the plates would be incubated at 37 °C for 30 min before measuring the absorbance at □ = 395 nm using a plate reader. The antioxidant capacity based on the ability to reduce ferric ions of the extract would be expressed as milligram $FeSO_4$ equivalents per hundred grams of sample extracts.

Statistical Analysis

Analysis of variance and significant differences among the mean of the triplicate data was performed by using two way ANOVA, SPSS (Version 18.0 for Windows, SPSS Inc., Chicago, IL). Data were expressed as means ± standard deviation and values $p < 0.05$ were regarded as statistical significant level.

RESULTS AND DISCUSSION

Total Phenolic Content (TPC)

Table 1 showed the total phenolic content in Manis Terengganu melon samples. The range of total phenolic content extracted with water, methanol and ethanol for all samples were in range of 12.69 to 1290.10 mg GAE/100 g. There were significant differences between all solvent used in order to extract the TPC. The TPC values that extracted by using the water was significantly higher compared to ethanol and methanol at all temperature (40 °C, 50 °C and 60 °C). These showed that the choice of the solvents used was one of the crucial parameters to extract the phenolic compound. Water have highest dielectric constant which mean the highest polarity of solvent followed by methanol, and ethanol. Thus, the greater the dielectric constant would give the best result of extraction (James, 2011). The increased of the solvents polarity caused the phenolic solubility increased (Yee et al., 2012). Muhamad et al. (2014) observed that solvent polarity play a vital role in increasing phenolic solubility. A polar solvent such as water will isolate polar compound which water-soluble (hydrophilic) antioxidants meanwhile non-polar solvent will extract non-polar compound which non-water soluble (lipophilic) antioxidant (Yee, 2012). Hence, these mean that Manis Terengganu melon contained high hydrophilic antioxidant.

In term of temperature drying, there was significant difference between extraction of TPC at 40 °C with 50 °C and 60 °C, but no significant differences of TPC values between 50 °C and 60 °C. The result showed that phenolic compound at 40 °C significantly the highest compared to 50 °C and 60 °C. High temperature with long drying time could destroy the phenols and decreasing water present in the plant cells, which makes the extraction with solvent more difficult (Jahangiri et al., 2011). Besides, it also reduce antioxidant value associated with thermal degradation of phytochemicals, enzymatic degradation of phenolic compounds and loss of antioxidant enzyme activities (Ee, et al., 2013).

Total Flavonoid Content (TFC)

Table 2 showed the total flavonoid content in samples of Manis Terengganu melon. The range of all samples in extract total flavonoid content was from 107.54 mg QE/ 100 g to 243.18 mg QE/ 100 g. Methanol showed the greater efficiency in extracting the flavonoids since it have significantly highest value compared to ethanol and methanol.

Table 1 Total phenolic content in samples

Total Phenolic Content (mg GAE/ 100 g)			
Temperature (°C)	Water	Methanol	Ethanol
40	$1290.10^{cB} \pm 33.02$	$145.84^{aB} \pm 25.99$	$412.14^{bB} \pm 40.13$
50	$249.87^{cA} \pm 47.26$	$25.17^{aA} \pm 25.98$	$91.75^{bA} \pm 24.97$
60	$220.74^{cA} \pm 28.83$	$12.69^{aA} \pm 7.21$	$162.48^{bA} \pm 79.28$

Each value was expressed as mean ± SD (n=3).

$^{a-c}$Means with different small letter within the same row are significantly different ($p < 0.05$).

$^{A-B}$Means with different capital letter within the same column are significantly different ($p < 0.05$)

These finding was similar with the finding by Dixon and Jeena, (2017) where methanol had the highest extraction value of flavonoids of Datura metel plant followed by distilled water, ethyl acetate and acetone. These result showed that the solvent use in the extraction influence by the polarity. This finding is also consistent with Boeing et al. (2014) who reported that methanol was the most effective solvent for extraction of antioxidant compounds, followed by water, ethanol and acetone. The polarities of methanol and ethanol are similar. High solvation of methanol as compare to ethanol might contribute to higher extraction yield (Boeing et al. 2014).

In term of drying temperature, there were no significant differences between temperatures 40, 50 and 60 °C. It is believed that the flavonoids content from Manis Terengganu melon are thermally stable up to 60 °C without leading to unfavorable degradation.

Table 2 Total flavonoid content in samples of Manis Terengganu melon.

	Total Flavonoid Content (mg QE/ 100 g)		
Temperature (°C)	Water	Methanol	Ethanol
40	$131.99^{a} \pm 3.61$	$243.18^{b} \pm 34.78$	$107.54^{a} \pm 17.02$
50	$155.70^{a} \pm 4.04$	$158.75^{b} \pm 10.61$	$140.48^{a} \pm 14.61$
60	$146.83^{a} \pm 19.13$	$182.55^{b} \pm 24.63$	$161.94^{a} \pm 19.25$

Each value was expressed as mean ± SD (n=3).

$^{a-b}$Means with different small latters within the same row are significantly different ($p < 0.05$).

DPPH Free Radical Scavenging Assay

Table 3 showed the amount of percentage of radical scavenging in samples of Manis Terengganu melon. The amount of percentage of radical scavenging range from 23.38% to 50.36% in all sample extracts using different solvent extraction (methanol, ethanol, distilled water). There was no significant difference between all solvents used. This finding was inconsistent with the study by Lee et al., (2018); Emad and Sanaa (2013) where methanol extract was found highest DPPH value followed by water and ethanol in watermelon rind and algae respectively. Vashisth et al. (2011) reported that phenolic compounds provide protection against harmful free radicals of edible and non-edible plant products. It also minimizes the risk of diseases associated with oxidative stress.

The antioxidant properties of plants were mainly influence by the phenolic compound presence in the plants (Lee et al., 2018). Boeing et al. (2014) stated that among the pure solvent for extraction of antioxidant compound, methanol was the most efficient followed by water, ethanol and acetone due to the better solvation of antioxidant compounds found in fruits. Meanwhile, ethanol showed the lower value radical scavenging activity compared to methanol even though have similar polarities, because of the existence of the ethyl radical in ethanol that is longer than methyl radical presence in methanol caused in lower solvation of antioxidant molecules.

The finding showed that there was a significant difference between drying temperature at 60 °C with 40 °C and 50 °C on the DPPH value of Manis Terengganu melon samples. This finding was consistent with the study by Hyeong et al. (2016) and Vallverdú-Queralt et al. (2014) where they conclude that high drying temperature significantly increased the content of total phenolic thus lead to higher radical scavenging activity. Heating has resulted in release of phenolic compounds from the cell wall, thus affects the bioaccessiblity of total phenolics (Tulipani et al., 2012). However, increasing temperature beyond a certain limit may induce degradation. Thus, 50 °C is the suitable temperature for drying Manis Terengganu melon.

Table 3 Percentage of radical scavenging activity

Radical Scavenging Activity (%)			
Temperature (°C)	Water	Methanol	Ethanol
40	$26.38^{A} \pm 3.02$	$23.38^{A} \pm 1.14$	$24.06^{A} \pm 3.23$
50	$47.96^{B} \pm 14.64$	$50.36^{B} \pm 9.31$	$47.47^{B} \pm 7.51$
60	$42.29^{B} \pm 4.37$	$49.30^{B} \pm 24.60$	$44.09^{B} \pm 11.27$

Each value was expressed as mean ± SD (n=3).

$^{A-B}$Means with different capital letters within the same column are significantly different ($p < 0.05$).

Ferric Reducing Antioxidant Power (FRAP)

Table 4 showed the amount of reduced ferric ions in samples of Manis Terengganu melon in order to determine its antioxidant capacity. It could be observed that the antioxidant activity for distilled water was significantly the highest followed by methanol and ethanol. These finding was consistent with the study reported by Sofia et al. (2012), which identified that the sample extract by water exhibited the higher FRAP value compared to the methanol and ethanol. Thus, highest antioxidant capacity of water extract measured by FRAP could be explained due to higher amounts of phenolic compound found in water extract. Sample extract using methanol solvent significantly higher than ethanol in reducing power due to high polarity of the solvent and stability of methanol solvent (Simon, et al., 2015).

The current finding showed that there was a significant difference between 40 °C with 50 °C and 60 °C for all extraction solvents. The Manis Terengganu melon sample dried at 60 °C exhibited the highest value of FRAP values. This finding was consistent with the finding by Ee et al. (2013) where the FRAP value at 60 °C of bitter guard melon showed the highest among the temperature used (40 °C, 50 °C and 60 °C). These might due to polyphenol oxidase (PPO) activity was fully activated at higher temperature (60 °C), and therefore the oxidation products from PPO contribute to the increased antioxidant capacities of bitter gourd fruit extracts.

Table 4 Value of reducing ferric ions in samples

Ferric reducing ions (mg Fe(II)/ 100 g)			
Temperature (°C)	Distilled water	Methanol	Ethanol
40	$97.90^{cA} \pm 12.28$	$188.46^{bA} \pm 110.31$	$32.07^{aA} \pm 30.50$
50	$516.64^{cB} \pm 7.73$	$140.69^{bB} \pm 47.19$	$97.22^{aB} \pm 22.36$
60	$594.31^{cB} \pm 28.15$	$231.58^{bC} \pm 43.29$	$99.29^{aB} \pm 7.30$

Each value was expressed as mean ± SD (n=3).

$^{a-c}$Means with different small letters within the same row are significantly different ($p < 0.05$).

$^{A-B}$Means with different capital letter within the same column are significantly different ($p < 0.05$).

CONCLUSION

The present study has demonstrated that drying temperature and type of solvent affect the extraction yield of total total phenolic, total flavonoid and antioxidants from Manis Terengganu melon. Drying temperature at 40 °C showed the highest value of TPC extract. Meanwhile, 50 and 60 °C exhibited higher value for antioxidant activity (DPPH and FRAP), respectively. Based on suitability of solvent extraction, methanol was the best solvent to extract the highest value of TFC as compare to water and ethanol. However, the used of water extract contribute to higher TPC and FRAP value. The present obtained result may be used in food industries for developing of functional food ingredient.

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