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BIOACTIVE CONTENT AND ANTIOXIDANT ACTIVITY OF ALBEDO POMELO (*Citrus grandis*, C. Maxima) EXTRACT USING A METHOD OF 2,2-Dhipenil-1-*PICRYLHYDRAZYL* (DPPH) IN VARIOUS TYPES OF EXTRACTION SOLVENT

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Abstract – Pomelo production has reached 110,000 tones annually and almost 50% albedo pomelo has not been fully utilized. Testing the phenol bioactive and flavonoid content of the albedo pomelo as well as their antioxidant activity is important. Determining the phenol total using the method of DPPH (*1,1-diphenyl- 2-picrylhydrazyl),* flavonoid total using quercetine as comparison and antioxidant activity of various types of extraction solvents by spectrophotometry UV vis. Sample was taken randomly using complete random design. Data obtained were analyzed with the analysis of variance using the F test. Young albedo extract has total phenol of higher than that of mature albedo extract, i.e., 2.04>1.94 g/100 g. Total flavonoid content of mature albedo extract is higher than young albedo extract, i.e., 0.18>0.10 grams/100 g. Ethyl acetate extract has the strongest antioxidant activity with the least IC value of 7.71 ppm compared to that of other solvents. The albedo pomelo extract contain phenolic bioactive compound as well as flavonoid, ethylacetate extract being the strongest in antioxidant activity.

INTRODUCTION

Pomelo fruit (*Citrus maxima* (Burm.)Merr) comprises of multiple nutritional components (Vijaylakshmi and Radha, 2015). Up to date, the albedo pomelo has not been optimized in utilization and regarded as wastes thus typically removed (Zamantha Escobedo-Avellaneda *et al.,* 2014). This waste problem must be overcame appropriately in order to reduce cost and prevent enviromental pollution.

Wastes from the albedo pomelo contain bioactives, *inter alia*, polyphenol, carotenoids, flavonoids and essential oils. Those bioactives act as natural antioxidants which is beneficial for health (Anagnostopoulou *et al.,* (2006); Levaj *et al*., (2009), Zulkifli *et al.,* (2012). The polyphenol comprised in albedo is higher than those in the other parts (Londono-Londono, 2010; Anagnostopoulou, 2006; Zulkifli *et al.,* 2012). Generally, albedo has the highest total phenolic content starting from 553.1 to 730.0 mg GAE/100 g (Zamantha Escobedo-Avellaneda *et al*., 2014). Albedo dried in the oven in 50 °C and 60 °C (892-1336 mg GAE/100 g) while if freeze-dried (555-1267 mg GAE/100 g) (Nur Farhana Abd Rahman *et al.,* 2018). Flavonoid is among phenolic compounds group which provides good source of phytochemicals and natural antioxidant (Xi, *et al*., (2014); Vijaylakshmi and Radha, (2013).

Based on the above, the objective of the present research is to test the phenol bioactive and flavonoid comprised in the albedo pomelo as well as their antioxidant using the method of2,2-diphenyl-1-*picryldhydrazyl* (DPPH) in various types of extraction solvents.

MATERIALS AND METHODS

(1) Preparing Sample of Albedo Pomelo Extract

a) Preparing Albedo powder

Albedo pomelo is subjected to drying by oven vacuum in temperature around 40 °C for 16 hours until material water content of 15± 0.5%. Size-reduced by pulverization using a grinder, subsequently screened using 80 Mesh sieved to obtain albedo powder.

b) Preparing albedo extract with Maceration Method

Albedo powder is introduced into a vessel and macerate in ethanol 96% (p.a) 1:10 while stirring for 24 hours in ambient temperature (darkened) for optimum extraction and the bioactives dissolved completely. The macerate obtained is filtered using the Whatman 42 paper to obtain filtrate.

c) Concentrating Macerate with Vacuum Rotary Evaporator

Macerate obtained from Maceration process, the solvent was removed with vacuum rotary evaporator. Concentrating with the rotary evaporator (Condenser cooler T:5 °C water bath T:40 °C vacuum pressure of 73 mbar) (recirculating chiller F-305; Rotavapor R-300; Vacuum Pump V-300; Heating B-300 Base). Concentrating with rotary evaporator in 40 °C until no ethanol is condensed (all of the ethanol has evaporated proven by no solvent dripping from round bottom flask) to give a thick extract. Freeze-drying 3x24 hours (ice condenser -50; vacuum 0.040 mbar, vacuum pump RZ 2.5; Freeze Drying Christ Alpha 1-4 LO plus). The obtained extract is introduced into an amber glass bottle and kept at cooler temperature (-25 °C). Extract of young and mature albedo is ready to be used for DPPH, phenolic and flavonoid total test.

2) *In vitro* Antioxidant test of Albedo

(a) Total phenolic assay using the DPPH method (Molyneux, (2004); Siddhuraju *et al.*, (2007); Jang *et al.*, (2010); Rafaela GuimaraÞes (2010); Chen *et al.*, (2012) 0.1-1.0 mL filtrate was pipetted, placed in 25 mL distilled water 0.5 mL of Follin Ciocalteu's (1:1) was added. Homogenized in 30 sec. Add 20% Na_2CO_3 2.5 mL, Subsequently homogenized. 25 mL of distilled water is added, subsequently incubated for 40 minutes in darkened room. The absorbance is measured on 725 nm.

Preparing standard Gallic Acid curve of 100 ppm. Procedure analogue with the assay for phenolic level was performed. Subsequently, DPPH test (Molyneux, 2004) by weighing the young and mature albedo. Diluted with 25 mL of methanol p.a. Pipetting the extract stock solution as follows:

All of the blending was performed in a reaction tube, subsequently incubated for 30 minutes in a darkened room. Absorbance read λ = 517 nm. Plotting the standard curve with percentage (%) of inhibition obtained. Calculate the IC_{50} value from linear regression curve between the percentage (%) of absorbance inhibition with various concentration of test solutions using the formula: % = (A_{blank}-A_{sample}) / A_{sample} x 100. After obtaining the percentage (%) of absorbance inhibition activity, the IC50 was sought using the linear regression equation of y = a + bx. *Radical Scavenging Activity* DPPH iscalculated using the formula:

$$\text{Radical Scavenging Activity (\%)} = \frac{\text{(1-Sample absorbance in 517 nm}}{\text{(Control absorbance in 517 nm}} \times 100$$

b) Total Flavonoid Assay in the Albedo Extract

Young and mature dried albedo extract. Weighing the dried albedo extract 0.1-1.0 grams. Placed in 25 mL methanol p.a (sample stock solution). Pipetting 1 mL of the sample stock solution, add with 3 mL of methanol p.a, 0.2 mL $AlCl_3$ 10%, and 0.2 mL of 1 M Potassium acetate. Add the distilled water until 10 ml. Homogenized for 10-3- seconds. Incubation for 30 minutes under ambient temperature in the darkened room. Measure with Spectrophotometry UV-vis in 431 nm.

Prepare quercetin standard curve (QE) of 50 ppm by dissolving it into the methanol (pa) until volume of 10 mL. Performing procedure analogue with the flavonoid assay procedure. Total flavonoid content is expressed as gram of quercetin equivalents per 100 grams sub-fraction (% w/w EK).

(3) Research Data Analysist

Data obtained if normally distributed and homogenous thus the data analysist technic being used in the research is parametric test, i.e., statistical test utilized for testing the true or false of null

Table 1. Sample Blending

Concentration (ppm)	Sample	Blank
Reference(R)	2 mL Methanol + 0.5 mL DPPH	2.5 mL Methanol
X ppm	2 mL sample + 0.5 mL DPPH	2 mL sample + 0.5 mL Methanol
X ppm	1 mL sample + 1 mL Methanol + 0.5 mL DPPH	1 mL sample + 1.5 mL Methanol
X ppm	0.5 mL sample + 1.5 mL Methanol + 0.5 mL DPPH	0.5 mL sample + 2 mL Methanol
X ppm	0.25 mL sample + 1.75 mL Methanol + 0.5 Ml DPPH	0.25 mL sample + 2.25 mL Methanol
X ppm	0.125 mL sample + 1.875 Methanol + 0.5 mL DPPH	0.125 mL sample + 2.375 Methanol

Table 2. Yield of Mature and Young Albedo

Sample	Initial Weight Albedo (g)	Weight Albedo Powder (g)	Rendement (%)
Albedo Mature	773.52	178.12	23.03
Albedo Young	432.97	98.28	22.70

hypothesis. In contrast, if the data is not normally distributed nor homogenous thus the data analysist technic being used in the research will be a non-parametric test.

Bioactive Content and Antioxidant Activity of Albedo Pomelo (*Citrus grandis, C. maxima*) Extract Using A Method of *2,2-dhipenil-1-picryldhydrazyl* (DPPH) in Various Types of Extraction Solvent

RESULTS AND DISCUSSION

Preparation of Albedo Powder

Results of preparation of young and mature albedo powder is as listed in Table 2.

The yield from mature albedo is higher that of the young albedo, and the mature albedo powder is heavier (178.12 grams) compare to that of the young albedo (98.28 grams). Mature albedo powder is deeper red than the young albedo which is lighter red since tannin content in mature albedo is higher than that of young albedo.

Test for Total Phenolic Content

Test result for mature and young albedo extract is as listed in Table 3.

Young albedo extract has higher total phenolic content compared to that of mature albedo extract.

Table 3. Mean value of Total Phenolic Content of Young and Mature Albedo Extract

Sample	Phenol (g/100 g)
Albedo Mature	1.94
Albedo Young	2.04

Determination of total phenolic content is performed to see the correlation between antioxidant activity and the total phenolic content. The total phenolic content is expressed by %w/w Gallic Acid Equivalents (% w/w GAE) (Siddhuraju, *et al*., (2007); Molyneux, 2004).

The use of gallic acid as standard solution is that the gallic acid bears moiety of hydroxyl and conjugated double bond by each benzene rings thereby the compound is easy to react to form a complex with the reagent Folin-Ciocalteu's and being component unit of the phenolic compound. Phenol is a compound having an aromatic ring with one or more hydroxyl moiety binds to the carbon atom of the aromatic ring. The hydroxyl moiety in phenol directly contribute to the antioxidant activity and play important role in capturing the free radicals since the hydroxyl moiety of the phenolic compound can donor hydrogen atoms so as to stabilize the free radical compounds (Rezaeizadeh, 2011).

The DPPH method is used for measuring the antioxidant capability of the albedo extract being a simple, fast and cost effective in measuring antioxidant capability in food, fruit and vegetables in quenching the free radicals. Basically, unpaired electron of the DPPF molecule give maximum absorbance at certain wave length, marked with purple color. The color will change from purple to light yellow when the unpaired electron is paired with hydrogen atom contributed by the antioxidant compound. The color changes is based on chemical equilibrium reaction (Prakash *et al*., 2012).

DPPH is a stable free radicals due to the resonance. The resonance also contributes in concentration of the purple color. When the DPPH solution is blended with a hydrogen atom donor compound, the DPPH will be reduced which is marked by reduced in purple (Molyneux, 2004).

Test for Total Flavonoid Content

Test result is provided in the Table 4.

Total Flavonoid content of mature albedo extract is higher than that of young albedo extract.

Table 4. Mean Value of Total Flavonoid Content of Young and Mature Albedo Extract

Sample	Flavonoid (gram/100 g)
Albedo Mature	0.18
Albedo Young	0.10

Flavonoid is a polyphenol compound which act as natural antioxidant. The high phenolic level in a material indicates high level of flavonoid in said material (Maisuthisakul, 2008).

Table 5. Antioxidant activity of Mature Albedo Extract

Solvent type	Concentration of the Extract (ppm)				
	200	275	350	425	500
	IC_{50}(ppm)				
Aq.dist	768.17	1393.22	727.89	1184.14	1281.31
Ethyl Acetate	39.95	35.39	34.58	11.99	7.71
n-Buthanol	1691.19	2217.27	2046.61	2266.90	2513.72
n-Hexane	2504.19	2532.12	3600.03	9124.09	9105.66

Determination of total flavonoid content conducted by using the spectrophotometry and expressed in %w/w quercetin equivalent (%w/w QE) serves in showing the relationship between antioxidant activities with flavonoid content. Quercetin is selected as comparative solution because it is one of the flavonoids which react with $AlCl_3$ to form a complex. Flavonoid has wide ranges of activities as natural antioxidant and metal chelators and related to activities such as anti-inflammatory, anti-allergy, hepatoprotective, anti-thrombosist, anti-viral and anti-carsinogenic.

Test for Antioxidant Activity in Mature Albedo Extract

The test result is provided in Table 5

At concentration of 500 ppm, the ethyl acetate extract shows the strongest antioxidant activity as shown by the least IC_{50} value of 7.71 ppm compared to that of other extracts. The higher the extract concentration, the least the IC_{50} value. Antioxidant activity ranked from the strongest based on the IC_{50} value are: ethyl acetate extract (IC_{50}= 7.71 ppm) is stronger than the distilled water extract (IC_{50}= 1281.31 ppm). The distilled water extract is stronger than n-buthanol extract (IC_{50}= 2513.72 ppm). N-buthanol extract is stronger than the n-hexane extract (IC_{50}= 9105.66 ppm). These results indicate that ethyl acetate extract of albedo pomelo has stronger antioxidant activity than vitamin E with IC_{50} value of 8.27 ppm. Vitamin E is natural antioxidant used as comparison.

CONCLUSION

Based on the search result and data analysist, it is concluded that :
(a) The albedo pomelo extract contain bioactives compounds of phenol and total flavonoids. (b) The ethyl acetat extract has the strongest antioxidant activity with the least IC_{50} value of 7.71 ppm compared to that of other extracts.

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