Microwave-Assisted Extraction of Phenolic Compound from Soursop Peel (*Annona muricata L.*) and Its Antioxidant Potential and SPF Measurement

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Abstract. Microwave-Assisted Extraction of phenolics compounds from soursop-peel (*Annona muricata L.*) was conducted at a fixed microwave power of 420 W with ethanol as the solvent of varying concentration (50%, 70%, and 96%) and extraction time (3 min, 5 min, and 7 min). The highest total phenolic content value was 2.37 mgGAE/gr of dried soursop-peel and was obtained at an extraction time of 7 min with an ethanol concentration of 96%. At this optimum parameter combination (7 min, 96%), the IC_{50} value of the extract was 78.05 μg/mL and classified as a strong antioxidant. While the Sun Protection Factor value was only 22.71 and it is categorized as having a fair-sun protection capability. The extraction time plays critical role on the contact time between solvent and the solid. Increasing extraction time from 3 minutes to 7 minutes in extraction using 96% ethanol, increased the TPC yield to more than 100%. While the increase of ethanol concentration from 50% to 96% only increase the TPC content by only 45 – 55%.

Keywords: Soursop peel, Microwave, Total phenolic, Antioxidant activity, SPF.

1 Introduction

The risk of skin damage due to the UVA and UVB radiation can be minimized by the application of sunscreen. Sunscreen can also slow the rate of skin aging, formation of wrinkles, and pigmentation [1]. Sunscreens used today are chemical products that may cause negative effect to our body. Bioactive compounds found in plant-species has the potential to be formulated as a safe alternative for sunscreen. The plant-parts that can be used as a sunscreen has some characteristics such as it contains antioxidative agents.

Soursop (*Annona muricata L.*) belongs to the Annonacea family. Its fruit has potential health benefits such as containing high antioxidants that is potential for cancer treatment, anti-bacterial agent, and beneficial for skin's health. Soursop fruit consists of 67% of consumables while the rest 33% is disposed as waste. The waste consists 20% of skin, 8.5% of seeds, and 4% of columella [2]. Various studies have found that soursop fruit contains vitamins (dominantly vitamin C) and high levels of minerals [3,4]. The phenolic acids, flavonoids, and tannins that are contained in the leaves, pulp, and seeds is a potential antioxidant [5]. Soursop peel is also known to have antileishmanial activity [6]. Therefore, soursop waste in the form of peels is a

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potential source for natural bioactive compounds. Phenolic compounds could be extracted through the conventional or non-conventional methods. However, conventional extraction based on thermal-extractive process requires a long extraction time and requires the use of extensive volume of solvents [7]. Microwave energy can provide the driving force for the extraction process. The use of microwave in extraction has several advantages namely fast extraction time, lesser use of solvent and higher yield [8].

The aim of this study is to extract the total phenolic compound from soursop peel by means of microwave energy and the use of ethanol as the extraction solvent. The effect of extraction time and solvent concentration on the total phenolic content (TPC) was investigated.

2 Material and method

2.1 Chemicals, materials, and apparatus

Apparatus. *Erlenmeyer, assay tube,* microwave, vacuum oven, bulb, vial, blender, spatula, digital scales, measuring flask, volumetric pipette, micro-pipette, aluminium foil, and UV spectrophotometer Thermo Scientific Genesys 10S, and FTIR Thermo Scientific Nicolet iS5.

Chemicals and materials. Soursop peel, distilled water, ethanol p. a. (Merck), magnesium powder (Merck), sodium carbonate p. a. (Merck), iron (III) chloride, hydrogen chloride (HCl), gallic acid, reagent Folin-Ciocalteu, and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

2.2 Sample Preparation

Soursop peel was cleansed with water. The washed peel was then oven dried at 50°C for 40 h to reduce the amount of moisture to less than 10%. The dry peel was then grinded using blender.

2.3 Extraction Process

The dry samples were mixed with ethanol in a clean erlenmeyer flask with a 1:10 solid to liquid weight ratio. Three different samples were prepared with varying ethanol concentrations of 96%, 70%, and 50%. Extraction was performed using the Microwave-Assisted Extraction (MAE) method at a power of 420 W with extraction time of 3, 5, and 7 minutes [7]. The liquid fraction was separated from solid fraction using filtering funnel. The obtained liquid was subjected to a vacuum oven at 45 °C and 180 mbar (0.2 atm) to evaporate the solvent and subsequently concentrating the obtained extract.

2.4 Fourier transform infra-red

The extract was tested qualitatively using FTIR Thermo Scientific Nicolet iS5 to identify the functional group in the soursop peel.

2.5 Determination of total phenolic compound

Gallic acid solution with concentration of 1000 ppm was transferred into volume of 0.5; 1.0; 1.5; 2.0 and 2.5 mL and were diluted with ethanol to volume of 10 mL to obtain concentration of 50, 100, 150, 200 and 250 ppm respectively. The Folin-Ciocalteu reagent of 0.5 mL was the added to 0.5 mL of each diluted gallic acid solution and let it rest for 3 minutes to homogenize. Afterward, 4 mL of 7.5% Na_2CO_3 solution was added and let it rest for 30 minutes at room temperature. The absorption at wavelength of 765 nm was measured using the UV-Visible Spectrophotometer Thermo Scientific Genesys 10S. The calibration curve was prepared from the data of absorbance and the concentration of the gallic acid solution [9].

Following the same procedures, the soursop peel extract was diluted to concentration of 1000 ppm. Folin-Ciocalteu reagent with volume of 0.5 mL was added into 0.5 mL of the soursop peel extract and allowed to rest for 3 minutes. It was followed by the addition of 4 mL of 7.5% Na_2CO_3 solution and allowed it to rest for 30 minutes at room temperature. The absorbance at wavelength of 765 nm of this solution was measured using the UV-Visible Spectrophotometer. The gallic acid concentration of the sample was calculated based on the calibration curve using the standard gallic acid solution. The total phenolic content of the soursop peel extract was calculated using equation (1) [10].

$$TPC = \frac{C \times V \times 10^{-3}}{m} \quad (1)$$

TPC = total phenolic content (mg/g)
C = gallic acid concentration (mg/mL)
V = volume of extract (mL)
m = weight of extract (g)

2.6 Determination of antioxidant activity

Fifty (50) mg of extract was dissolved in 50 mL of ethanol p.a. in measurement flask to obtain a standard solution with concentration of 1000 ppm. The solution was then transferred into separate bottles with volume each of 0.2; 0.4; 0.8; 1.6; and 3.2 mL and were subsequently diluted with ethanol p.a. to volume of 10 mL. The obtained samples were diluted to 20, 40, 80, 160 and 320 ppm. Three millilitres of each sample were transferred to an assay tube and 4 mL of 0.4 mM DPPH solution was added [11]. The solution was stirred using a vortex for 30 seconds. The samples were allowed to homogenize and were stored in a dark condition for 30 minutes. The absorbances were measured by UV-Vis spectrophotometer at a wavelength of 515 nm.

Antioxidant capacity of the samples were determined through the calculation of inhibition percentage of DPPH absorption by using equation (2). A calibration curve was identified from the plotting of %inhibition percentage against concentration. From the value of a (the slope) and the value of b (the intercept) of the graph, the value of IC_{50} was obtained using equation (3).

$$\%Inhibition = \frac{\text{Blank Absorbance} - \text{Sample Absorbance}}{\text{Blank Absorbance}} \times 100\% \quad (2)$$

$$IC_{50} = \frac{50 - b}{a} \quad (3)$$

2.7 Determination of Sun Protection Factor (SPF) value

The SPF value was calculated by determining the area under the curve (AUC) from the absorbance value at a wavelength of 290 - 400 nm with an interval of 5 nm. The AUC value can be determined using equation (4):

$$AUC = \frac{A_a + A_b}{2} \times dP_{a-b} \quad (4)$$

Aa = absorbance on wavelength of a nm
Ab = absorbance on wavelength of b nm
dP_{a-b} = wavelength difference of a and b

The SPF value of each concentration was determined using equation (5) [12]:

$$\log SPF = \frac{AUC}{\lambda_n - \lambda_1} \quad (5)$$

λ_n = maximum wavelength (400 nm)
λ_1 = minimum wavelength (290 nm)

3 Results and discussion

The extraction was performed through microwave-assisted extraction which can reduce the extraction time because the samples were heated directly by microwave radiation in which the temperature increased at a higher rate [13]. The solvent used was ethanol because it can dissolve both less polar and polar compounds. It also has a high dielectric constant to absorb microwave energy. Ethanol is also low in toxicity, and it is safer to use as a formulation in sunscreens.

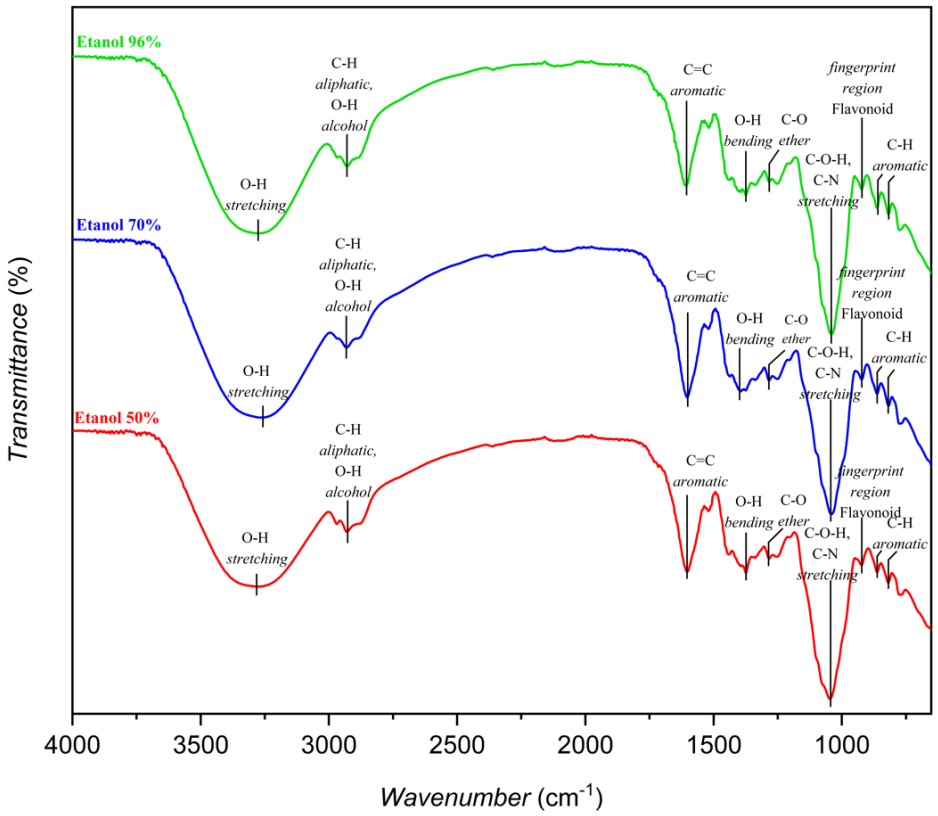

Fig. 1. FTIR spectrum of soursop peel extract.

Based on the FTIR result in **Figure 1**, the peaks show indication of the presence of functional groups of phenolic compounds, such as O-H, C-H, C=C aromatic, C-O and C-N groups. Flavonoid compounds are identified by the presence of aromatic C=C bond, -OH, C-H, C-O ether, and fingerprint areas. The FTIR results also indicated the presence of -OH rings, C-H stretching, aromatic C=C, and C-O ether which are the characteristics functional group of tannin-compound. While the presence of alkaloids is characterized by a C-N group at wavenumber of 1020-1250 cm^{-1}. The wavenumber of the functional groups was summarized in Table 1.

Table 1. FTIR identification of soursop peel extract.

Functional group	Wavenumber range (cm^{-1})	Wavenumber of soursop peel extract (cm^{-1})		
		50%	70%	96%
-OH *stretching*	3200 – 3550	3280,06	3259,69	3279,10
C-H *stretching aliphatic*	2850 – 2990	2929,89	2931,59	2929,63
C=C *aromatic*	1440 – 1625	1602,81	1602,60	1606,87

O-H *bending*	1310 – 1390	1374,06	1394,35	1373,62
C-O *ether*	1000 – 1300	1283,53	1283,98	1250,95
C-O-H	1000 – 1050	1046,93	1039,81	1039,70
C-N *stretching*	1020 – 1250	1046,93	1039,81	1039,70
Finger-print Flavonoid	900 – 1300	925,66	922,28	922,78
C-H *bending aromatic*	680 – 900	864,91	862,67	862,90
C-H *bending aromatic*	680 – 900	819,20	819,59	818,31

Gallic acid standard solution was used to determine the total phenolic content in the extract quantitively. The reaction of gallic acid with Folin-Ciocalteu reagent produces a blueish solution ($(PMoW_{11}O_{40})^{4-}$) which absorbs light with wavelength of about 765 nm [14]. The calibration curve of gallic acid standard solution with a concentration 50 – 250 mg/L was prepared. Then, the regression equation was derived as:

$$y = 0.0014\ x + 0.764 \quad (6)$$

Equation (6) with $R^2 = 0.99$ can be used to determine the total phenolic content of the extract. **Figure 2** shows that extraction time has the positive effect on the concentration of the obtained total phenolic compound. Longer extraction time facilitated longer contact time of the solvent to the solid surface, thus increasing the extraction rate. TPC concentration obtained at optimum extraction time (7 minutes) was 2.37 mgGAE/gr, 1.96 mgGAE/gr, and 1.58 mgGAE/gr for ethanol solution of 96%, 70%, and 50% respectively.

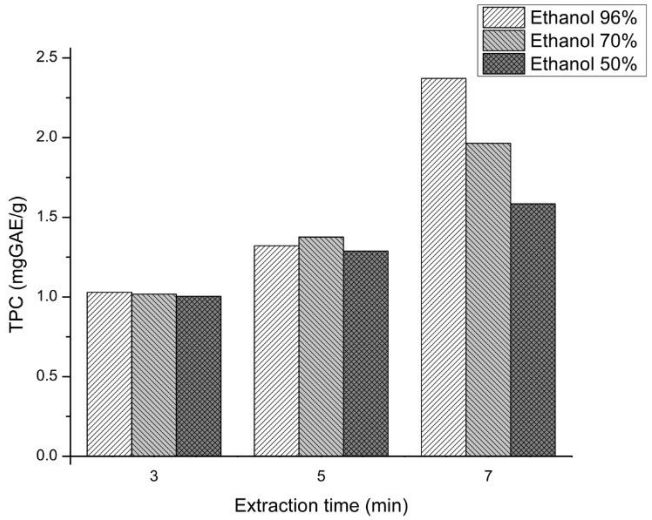

Fig. 2. Total phenolic content of the extract by variation of ethanol concentration and extraction time.

Standard error of measurement was determined using the Cronbach's Alpha method in which the reliability and alpha value were 0.80 and 0.05 respectively. Using 95% confidence level, the

total phenolic compound extracting using 96% ethanol for 7 minutes were 2.20 – 2.54 mgGAE/gr. While extraction using 96% for 5 minutes and 3 minutes resulted the TPC of 1.04 - 1.60 mgGAE/gr and 0.92 – 1.13 mgGAE/gr respectively. All in all, the total phenolic compounds values measured in experiment were within the value with 95% confidence level as shown in Table 2. The standard error of measurement serves in a complementary role to the reliability coefficient. If the test is reliable, the standard error of measurement (SEM) is at its minimum. When the test is completely unreliable, the standard error of measurement is at its maximum, equal to the standard deviation of the observed scores. The SEM of the experiment data were lower than the observed standard deviation. Thus, the experiment data were deemed reliable.

The data also shows increasing extraction time from 3 minutes to 7 minutes in extraction using 96% ethanol, increased the TPC yield to more than 100%. While the increase of ethanol concentration from 50% to 96% (extraction time of 7 minutes) only increase the TPC content by only 45 – 55%.

Table 2. Standard error of TPC measurement.

Ethanol concentration (%)	Extraction time (minutes)	TPC (mgGAE/gr)		Standard Deviation	Standard Error of Measurement (SEM)	TPC (mgGAE/gr)	
		Single	Duplo			Upper Limit	Lower Limit
50	3	1.05	0.96	0.07	0.03	1.06	0.95
	5	1.29	1.28	0.01	0.00	1.29	1.28
	7	1.54	1.63	0.07	0.03	1.64	1.52
70	3	1.07	0.97	0.07	0.03	1.08	0.96
	5	1.42	1.33	0.07	0.03	1.43	1.32
	7	2.08	1.84	0.17	0.08	2.11	1.81
96	3	1.11	0.94	0.12	0.05	1.13	0.92
	5	1.55	1.09	0.32	0.14	1.60	1.04
	7	2.24	2.51	0.19	0.09	2.54	2.20

Based on data of the solubility of gallic acid at a temperature of 313.15 K [15], the solubility of gallic acid as the standard testing solution in ethanol of 95% concentration is almost 50% higher than in ethanol 70% and 50%. Thus, it was expected at the same extraction time, the extracted-TPC from extraction using 96% ethanol will be higher. However, at a short extraction time, the extracted TPC content is almost the same. The effect of higher solvent concentration is more pronounced when coupled with a longer extraction time.

The method used in testing antioxidant activity was based on the light absorption of DPPH radical. DPPH solution displays a deep purple colour after dissolved in 99.9% ethanol due to the presence of a chromophore group of DPPH radicals that absorb strongly at a wavelength of 515 nm. Antioxidant capacity of soursop peel extract expressed in percent inhibition against DPPH concentration. In this study, extract solutions with concentration of 20, 40, 80, 160, 320 ppm were prepared, and when the DPPH solution was added into, the reaction caused the dark

violet colour changes gradually to pale yellow. The higher TPC concentration of the extract solution, the stronger the antioxidant activity, the solution became fader and exhibited smaller absorbance value. This is because hydrogen atoms bind to DPPH which indicates antioxidant activity in capturing free radicals. The value of antioxidant activity is represented by IC_{50} that indicates the required concentration of extract solution to inhibit 50% of the initial DPPH free radicals.

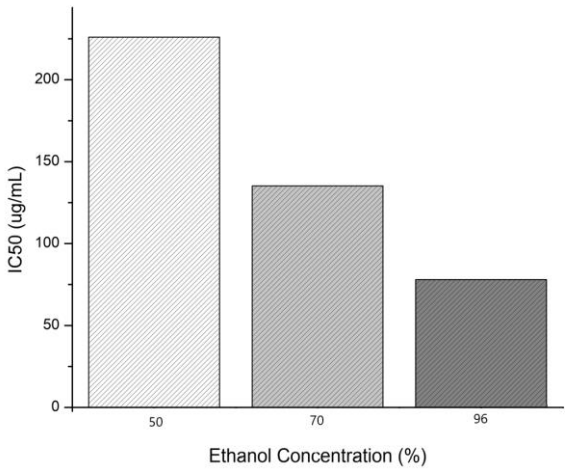

Fig.3. IC_{50} value of the extract by variation of ethanol concentration at optimum extraction time.

The IC_{50} value of the extract solution by ethanol 96% is 78.05 µg/mL. Whereas the IC_{50} value from the extraction with ethanol 70% is 135.22 µg/mL, and the IC_{50} from extraction using ethanol 50% is 225.99 µg/mL (**Figure 3**). The lower the IC_{50}, the higher the antioxidant activity. The antioxidant activity can be categorised as weak, moderate, and strong for extraction using ethanol 50%, 70%, and 96% respectively [16]. The higher antioxidant activity in extract solution from extraction using ethanol 96% due to the higher total phenolic content.

The SPF value refers to the strength of sunscreen to reduce erythema when skin is exposed to UV radiation. In this study, SPF tests were carried out on each extract from optimum extraction time (7 minutes) by employing the UV-Vis technique. The wavelength used is in the range of the UV-A spectrum (wavelength 400-315 nm) and UV-B rays (wavelength 315-290 nm). By using the equations (4) and (5), results were obtained as shown in **Figure 4**.

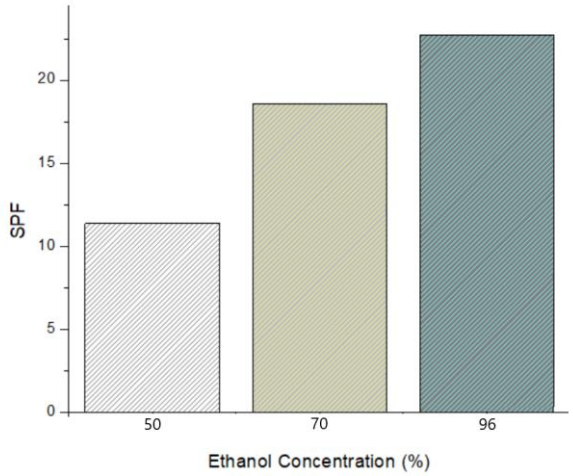

Fig.4. SPF value of the extract by variation of ethanol concentration at optimum extraction time.

The results of the study indicated that the higher the concentration of the solvent, the higher the SPF value. The SPF value is proportional to the TPC content. The existence of phenolic compounds has potential to be a photoprotective in sunscreen application. The largest compounds in the phenolic group are phenolic acids, flavonoids, and polyphenols with high molecular weight. Phenolic compounds have a hydroxyl group in the aromatic ring which contributes to the absorption of ROS (Reactive Oxygen Species) and the presence of a double bond can provide a high ability to absorb UV rays. The SPF value of soursop peel extract belongs to the low and medium protection based on SPF category [17].

4 Conclusion

The results obtained in this study show that higher solvent concentration enabling the greater phenolic compounds that were extracted from soursop peel. Also, the longer extraction times result in an increasing quantity of phenolic compounds. The extraction using ethanol 96% and extraction time of 7 minutes resulted in extract with the highest phenolic content 2.37 mgGAE/gr. All the TPC measurement data were within the 95% confidence level for TPC value. Furthermore, the standard error of measurement (SEM) of the data were lower than the observed standard deviation. Thus, the experiment data were deemed reliable. Using optimal extraction time (7 min), the strongest antioxidant activity of the extract with IC_{50} value is 78.05 μg/mL was obtained by extraction using ethanol 96%. The highest SPF value of the extract was 22.71 that was extracted using ethanol 96% and extraction time of 7 minutes.

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Author's contribution. Amanda Suzeta contributed in the development of research and methodology, experiment and data analysis,. Wegik Dwi Prasetyo contributed on the data analysis and manuscript editing. Agung Nugroho contributed on the manuscript editing. Rinaldi Medali Rachman contributed on the manuscript editing. Eduardus Budi Nursanto contributed on the manuscript editing.

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