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Determination of Polyphenol Content in Sawo Fruit (*Manilkara zapota*) Based on Geographical Location

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Abstract

Background: Sawo fruit (*Manilkara zapota* (L.) P.Royen) is rich in antioxidant compounds like polyphenols, and has long been used to treat diarrhea and thypoid by natives of Toari and Langori villages of Kolaka district of Southeast Sulawesi Province. Both villages located at different geographical location according to their altitudes from the sea level. The polyphenols content of sawo fruit from these villages that has a correlation with its antioxidant activity has yet investigated and thus need more research. Objective: This study was aimed to determine the content of polyphenols in sawo fruit based on geographical growth difference, that are Toari and Langori villages. Material and Methods: The fruits were collected from two locations of the Kolaka district that are Langori and Toari villages. The polyphenols content in the methanol extract of Sawo fruit was determined qualitatively using $FeCl_3$ and quantitatively using the Folin-Ciocalteu reagent measured by UV-Visible spectrophotometry. Gallic acid was used as the standard polyphenol of the assay. Results: The polyphenols content of sawo fruit from Langori found to be 1.48113 mg/g, while fruits from Toari contained 1.55747 mg/g of polyphenolics. Conclusion: The study showed that there was an influence of the geographical growth on the content of polyphenolics of sawo fruits.

Keywords: Sawo fruit, Manilkara zapota, Polyphenols

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1. Introduction

Manilkara zapota is a tree native to the Americas and the Atlantic Ocean. The plant has been internationally named as sapodilla and its fruit is popular in Indonesia as buah sawo. The sweet and meaty mustard fruit of sawo is soft when ripe and rich in calories, which contains fructose and sucrose. According to Gomathy [1], sawo is one of the plants that rich in antioxidant compounds. The fruit contains polyphenols, carotenoids, saponins, flavonoids and tannins that can act as antibacterial agent [2]. The polyphenol content of the fruit is able to overcome oxidative stress due to its antioxidant effects [3].

Sawo trees are often found in two villages of Kolaka district, Southeast Sulawesi, that are Toari and Langori villages. In addition to being a source of nutrition, people in both villages have been using sawo fruit as folk medicine to treat diarrhea and thypoid. Based on the geographical altitude, Toari village is located about six meters above the sea level (6 m above sea level) with the coordinate points at 121° 28'00''E - 121° 37'00''E and 4° 31'00''S - 4° 35'00''S, while Langori village is located about twenty-nine meters above the sea level (29 m above sea level) and coordinate point at 121°46'30''E - 121°37'30''E and 4°08'00''S - 4°12'15''S. Therefore, there are differences of weather and microclimate, especially temperature and humidity between Toari and Langori villages [4]. The region differences also affect the contents of compounds and pharmacological activities of plants among species [5]. As far as our concern, study of polyphenol content in sawo fruits from Toari and Langori villages has yet investigated. Therefore, in this article, we evaluated the polyphenol content of sawo fruit from both villages using UV-Visible spectrophotometric methods.

2. Experimental section

2.1 Materials

Apparatus and instruments used in this study include separatory funnels (Pyrex®) Erlenmeyer 250 mL (Pyrex®), Beaker (Pyrex®), measuring glass 100 mL (Pyrex®), volumetric flask 100 mL and 250 mL (Pyrex®), measuring pipette 1 mL, 10 mL and 50 mL volume pipettes, cutting blades, Blender (Philips®), rotary evaporators (Buchi®), UV-VIS spectrophotometers (UV-Mate®), test tubes (Pyrex®), and analytical scales (Ohaus®).

The chemicals used are gallic acid (Sigma-Aldrich®), $FeCl_3$ (Sigma-Aldrich®), ethanol 96% (PT. Brataco), methanol 96%, sodium carbonate (Sigma-Aldrich®), Reagent Folin-Ciocalteu (Sigma-Aldrich®). Meanwhile, Whatman No.4 filter paper is used to filter the extract.

2.2 Sampling

Samples of sawo fruits (*M. zapota*) were taken 1 kg each from 2 villages in Kolaka district, Southeast Sulawesi, namely Langori and Toari villages (Figure 1). The samples were authenticated by the *Dinas Tanaman Pangan & Hortikultura Kolaka* with voucher numbers of MZ-0001 and MZ-0002 for sawo fruits from Langori and Toari villages, respectively.

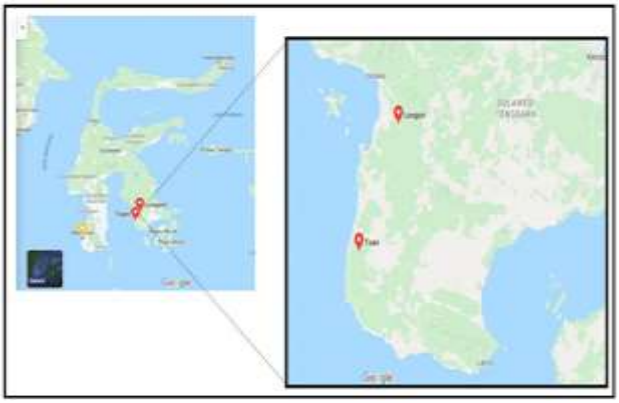

Figure 1. Map of the sampling location.

2.3 Sample Preparation

Fresh-peeled fruits of sawo each from Toari and Langori villages were weighed 250 grams and blended for 3 minutes. The sample was further macerated with 250 mL methanol for 3 hours with periodically shaking. The maceration process was repeated 3 times. The macerate was filtered using Whatman filter paper to get the filtrate and then concentrated using a rotary evaporator to yield the extract of sawo fruit.

2.4 Preparation of Folin-Ciocalteu Reagent

The Folin-Ciocalteu's reagent was prepared by dissolving 100 g of sodium tungstate, 25 g of sodium molybdic, 700 mL of aquadest, 50 mL of phosphoric acid, and 100 mL of hydrochloric acid in a 1000 mL boiling flask. The mixture is refluxed for 10 hours, added 150 mL of lithium sulfate, and 50 mL of Br_2 solution. The mixture was refluxed again for about 15 minutes and let cooled at room temperature. Then, the mixture was diluted with distilled water to reach a volume of 1 L reagent solution.

2.5 Na_2CO_3 20 % (w/v)

The Na_2CO_3 solution (20%, w/v) was prepared by dissolving 5 g of Na_2CO_3 in 20 mL of distilled water, then the solution boiled. The solution was kept for 24 hours, filtered, and diluted with distilled water up to 25 mL of solution [6].

2.6 Qualitative Analysis

Two-mL of viscous extract of sawo fruit was transferred into a test tube and added with $FeCl_3$ solution. The formation of a greenish-black color indicated the presence of phenol compounds in the extract [7]. A sample contains phenols when observed as green, red, purple-blue, or black by using the $FeCl_3$ reagent [8].

2.7 Quantitative Analysis

2.7.1 Preparation of gallic acid standard solution

Precise amount of 50 mg of gallic acid was used to make stock solution of the polyphenol standard. A 1000 ppm gallic acid stock solution was prepared by dissolving gallic acid in 5 mL of 96% ethanol and added with distilled water up to 50 mL in a volumetric flask. Then, the standard solutions of gallic acid were made in serial concentrations of 2, 4, 6, 8, and 10 ppm [6].

2.7.2 Determination of the maximum wavelength

A standard solution of gallic acid (6 ppm) pipetted 0.5 mL into a vial and then added with 15.5 mL of distilled water and 1 mL of Folin-Ciocalteu reagent. The mixture was incubated for 2 hours at room temperature and then measured at a wavelength range of 400-800 nm. The wavelength indicating the highest absorbance value is the maximum wavelength [6].

2.7.3 Determination of the gallic acid calibration curve

The standard solutions of gallic acid (2 to 10 ppm) pipetted 0.5 mL into each vial and then added with 15.5 mL of distilled water and 1 mL of Folin-Ciocalteu reagent. The mixture homogenized and then let stand for 8 minutes. The reaction was stopped by adding 3 mL of Na_2CO_3 20% solution and the mixture incubated for 2 hours at room temperature. The absorbance of the mixture was then measured at 666 nm. A linear calibration curve of gallic acid was plotted using the measured absorbances versus concentrations [6].

2.7.4 Determination of the polyphenol content of the sample using the Folin-Ciocalteu method

The methanol extract of sawo fruit weighed 0.3 g and dissolved in 10 mL of methanol-water (1:1). The solution pipetted 0.5 mL into a vial and added with 15.5 mL of distilled water and 1 mL of Folin-Ciocalteu reagent, following by incubation for 8 minutes. After incubation, the mixture was added with 3 mL of Na_2CO_3 20%. The reaction mixture was incubated again for 2 hours at room temperature and the absorbance measured at 666 nm against a blank containing only sample and solvent. The experiment was repeated three

times. The absorbance of sample was interpolated into a linear calibration curve of the gallic acid. The polyphenol content in the sample was presented as milligrams of gallic acid equivalent per gram of fresh sample a(mg GAE/g extract) [6].

2.8 Data analysis

Data were obtained from three replicates and presented as mean ± standard deviation (SD). Data analysis was carried out using GraphPad Prism 5 Software (GraphPad Inc., California, USA). The difference between polyphenol content from Toari and Langori villages was compared using Analysis of Variance (ANOVA) with t-test ($p < 0.05$).

3. Results and Discussion

The use of sawo fruit in traditional medicine by natives of Toari and Langori villages has made the plant a favor for cultivation. Thus, sawo is found growths in almost every yard of the house, because of the belief as a beneficial medicinal plant. According to Priya *et al.* [9], antimicrobial and antioxidant activity exhibited by sawo is related to its content of polyphenols. In the present study, sawo fruits were obtained from Langori Village and Toari Village, Kolaka Regency, Southeast Sulawesi. Samples were extracted by the maceration technique. This technique prevents compounds decomposition, especially polyphenols when compared with other methods that apply heating like soxhlet and reflux [10]. The use of methanol in the maceration of sawo fruit has correlated with the favor solubility of polyphenols in this solvent, increasing the yield of extraction of polyphenols from the fruit [11]. In this study, the yields of methanol extract of the fresh fruit from Toari and Langori villages were 301.1 and 301.7 mg, respectively (Table 1). The yields were not significantly different which is about 0.12% of 250 g fresh fruits. Results indicated high water content in the fruit of sawo.

Table 1. Results of qualitative analysis of sawo fruit polyphenols based on geographical location using $FeCl_3$ reagent

Sample Name	Sample Code	Extract weight (mg)	Observation	Indication
Sawo fruit from Langori	BS1	301.1	Dark green	Positive
Sawo fruit from Toari	BS2	301.7	Dark green	Positive

The qualitative analysis of methanol extract of sawo fruit using the reagent $FeCl_3$ showed a greenish-black color (dark green) in the sample solution, indicating the presence of polyphenols (Table 1). Polyphenols have antioxidant activity that is beneficial for the treatment of diabetes, mutagenesis, heart diseases, inflammation, and cancers [12]. The antioxidant activity of phenolic compounds has been correlated to the formation of phenoxide ions that provide one free electron to free radicals [13]. The role of antioxidant phenolics in cells is to suppress gene mutations rate in cells and reduce inflammation [12]. In addition, the unripe fruit of sawo has shown antibacterial activity against *Salmonella thypii* and *Escherichia coli* due to its polyphenols content [14]. Compounds belonging to the polyphenol group have been reported in promoting bacterial cell walls and membranes contraction as wells as growth proteins decomposition. The process has resulted in bacterial growth inhibition, membrane cell damage, and peptidoglycan components destruction which leads to cell death [2]. Determination of polyphenols content with Folin-Ciocalteu method showed different levels between sawo fruit grown in Langori and Toari Villages, indicating the influence of growing area factors. According to Pangestu *et al.*, the soil of the Langori village geographically consists of a laterite-type structure. The laterite-type soil contains iron and aluminum with low fertility rate, low nutrients content, and less water availability [15]. Meanwhile, the soil structure of the Toari village is a podsolic-type that generally has lower fertility and nutrients content as compared to other soil structures [16]. Previous studies have shown that the soil

structure and condition contributed a significant role to the polyphenols content in plants. Besides, the growing area factors, such humidity, air temperature, and nutrients influenced the biosynthesis of secondary metabolites by plant species, including polyphenols [7]; [17]. Plant secondary metabolites have vital protective roles against environmental stress, plant diseases caused by pathogens (fungi, bacteria, and virus), herbivores as well as competing with other plants [18]. The different polyphenols content of sawo fruit from Toari and Langori villages was suggested due to the low nutrient soil conditions and low fertility levels as factors of the environmental stress. Polyphenols are chemical compounds that can support the human body system to fight several health problems and cell aging. Structurally, compounds belong to polyphenols contain more phenol groups as compared to other groups like steroids and triterpenoids. For the plant itself, polyphenols are produced for defense towards ultraviolet radiation and pathogen aggression, as well as facilitating attractive colors to draw the attention of beneficial small-scale animals like pollinators and seed spreaders. The fruits, including sawo fruit, are a source of natural antioxidants and contain lots of phenolic compounds (polyphenols) which is considered as the nutritional content and benefits of sawo fruit for health [12].

Table 2. The content of polyphenols in sawo fruits is based on geographical location in UV-Visible spectrophotometry at wavelengths of 666 nm

Sample	Absorbance (A)	Content in the sample (mg GAE/g)	Sample mean concentration (mg GAE/g)	P value
BS 1	0.176	1.47	1.48 ± 0.01	p<0.0001
	0.178	1.49		
	0.178	1.49		
BS 2	0.183	1.54	1.56 ± 0.02	
	0.185	1.56		
	0.187	1.58		

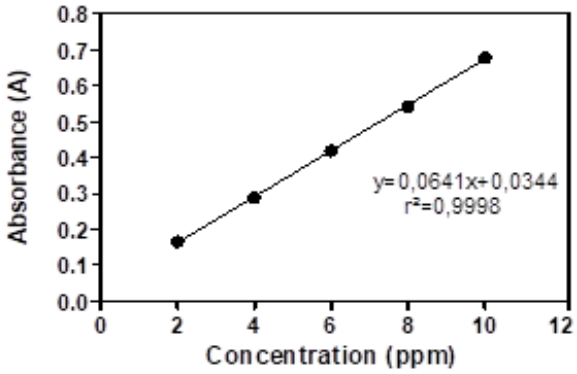

Figure 2. Gallic acid calibration curve

4. Conclusion

In a qualitative analysis of both sawo fruit samples based on their geographical location, positive contains polyphenol compounds characterized by the addition of greenish-black color after the addition of $FeCl_3$. In a quantitative analysis using UV-Vis spectrophotometry at wavelength 666 nm obtained results, sawo fruit from Toari village has a polyphenol content of 1.55747 mg/g, while sawo fruit from Langori Village has a polyphenol content of 1.48113 mg/g. The study suggested that geographical location influences the polyphenol content of sawo fruit.

Conflict of Interest

No conflict of interest is associated with this study.

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