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RESEARCH ARTICLE

Evaluation the Fractions from Extract Ethanol Psidium Guajava Leaves on Total Phenolic Content, Antioxidant Activity and Its Antibacterial Activity of Staphylococcus Aureus

Mustofa Ahda[1,2*], Ririn Lestari Sri Rahayu[1], Wahyu Tika Lestari[1], Rizki Dwi Mantoro[1]

1. *Faculty of Pharmacy, Universitas Ahmad Dahlan.*
2. *Ahmad Dahlan Halal Centre, Universitas Ahmad Dahlan.*

***Corresponding Author: Mustofa Ahda**

Abstract

Objective: The aims of this research to find the potential extract and fraction from ethanol extract of Psidium Guajava L for natural antioxidant and antibacterial. The extract and fraction of Psidium Guajava L was analyzed the phenol total content, antioxidant activity and its antibacterial. Methods: The ethanol extract of Psidium Guajava L was extraxted using n hexane, ethyl acetate and water. The all fraction and ethanol extract of Psidium Guajava L was determined the scavenging activity, penolic compound. The scavenging activity was determined by DPPH method. The phenolic compound was determined with Folin-Ciocalteu method. Results: The result of this research showed that the fraction of ethyl acetate has highest antioxidant activity with the value is 9.07 µg/mL. It is also supported by the result of screening phytochemical that ethyl acetate fraction contains mostly complete of chemical substances. However, The highest total phenol content was found in water fraction with phenolic content of 26%. The anbacterial activity of ethanol 96% extract of Psidium guajava leaves and ethyl acetate fraction have a highly activity to inhibit the Staphylococcus Aureus. Conclusion: The fraction of ethyl acetate has a highest of antioxidant activity, although it doesn't have highest of total phenolic content. The highest of total fenolic content from water fraction is 26%. The scheme of antibacterial activities potential of Guajava extracts are ethanol 96% extract = ethyl acetate fraction > n-hexane fraction = water fraction.

Keyword: *Antioxidant, Antibacteri, Total phenolic content, Ethyl acetate fraction.*

Introduction

The plant of Psidium guajava L. is used in some of health problem solutions both of its fruit and its leaves. The fruit and leaves of Psidium guajava L is used as anti diarrhea. Therefore, this plant has potential to develop as pharmaceutical dosage. Reference [1] said that some of parts of Psidium guajava L. plant were used in developing some industry and pharmaceutical products.

Event, its leaves has some of active compound such as flavonoid that is quercetin, quercetin-3-O-glucopyranoside and morin in methanol extract from guajava leaves [2]. Based on reference [3] reported there are 45 and 42 compounds in the fruit and oil of Psidium guajava L. The research was reported by reference [4] that essential oil from the leaves of Psidium guajava L. has moderate antioxidant activity to DPPH. The rich of this compounding underlies to make fractionate process to get potential active compounding in the leaves of Psidium guajava L. There are 3 flavonoid groups as active compound in the leaves [2].

Besides that, the leaves of Psidium guajava L. contains active compound that are tannins group, coumarin [5], phenols, chlorophyll and carotenoids [6], tannins, terpenoids, steroids, glycosides, cardiac glycosides, phlobatannin, alkaloids and reducing sugars and absence of saponin and anthraquinone [7]. The purpose of fractionation is increasing antioxidant activity by losing substance that has not antioxidant activity

Materials and Methods

Materials

The raw materials were guajava leaves (Psidium guajava L.) that took in Yogyakarta, ethanol 96%, 2,2-difenil-1,1-pikrilhidrazil (DPPH) p.a. (E-Merck), ethyl acetate (E-Merck), n-hexane (E-Merck), standart quersetin (Sigma-Aldrich), Mayer reagent, Dragendorff reagent, HCl (E-Merck), Mg powder (E-Merck), $FeCl_3$ 1%, Metilen blue (E-Merck), alcohol absolute (E-Merck), HNO_3 concentrated (E-Merck), Other chemicals were distilled water, Na_2CO_3 7.5%, Folin Ciocalteu Reagent (E-Merck), Gallic acid (Sigma-Aldrich). Staphylococcus Aureus bacteria and (BHA)

Sample Preparation

The leaves of Psidium guajava L. were washed and dried in the oven at 50 ^{0}C for 24 hours, and then powdered using a blender. Powder was filtered using mesh No. 30 to control the particles size of leaves of Psidium guajava powder.

Extraction of Leaf of Psidium Guajava L

200 gr powders were macerated with ethanol absolute 96% with stirred for 24 hours and after that, the solution was filtered using a vaccum and separated between the pulp and filtrate. Filtrate was collected in the bottle as macerate I. Pulp was re-macerated with ethanol absolute 96% as like step before to obtain macerate II. Macerate I and II were collected and condensed using rotary evaporator at 70^{0}C, then were dried to obtain the viscous extract. Viscous extract was weighed.

Fractionation of Ethanol Extract of Psidium Guajava L

20 gr ethanol extract of Psidium guajava L was dissolved in aquades 20 mL, n-heksan 40 mL, stirred and hushed till appear two parts and then was separated. The dissolve part was taken by separating funnel (two fractionation). Filtrat was collected and condensed at low pressure and low temperature at about 50^{0}C.

The solid part is fractional that undissolved in n-hexane. Fraction that undissolved in n-heksan was refractionated use ethyl acetate. The aquades were added 10 mL, and ethyl acetate 20 mL. This process would have fraction that dissolve in ethyl acetate and fraction that undissolved in ethyl acetate as water fraction. Both of fractions were condensed on waterbath till viscous mass was formed. The scheme of Fractionation process as Figure 1.

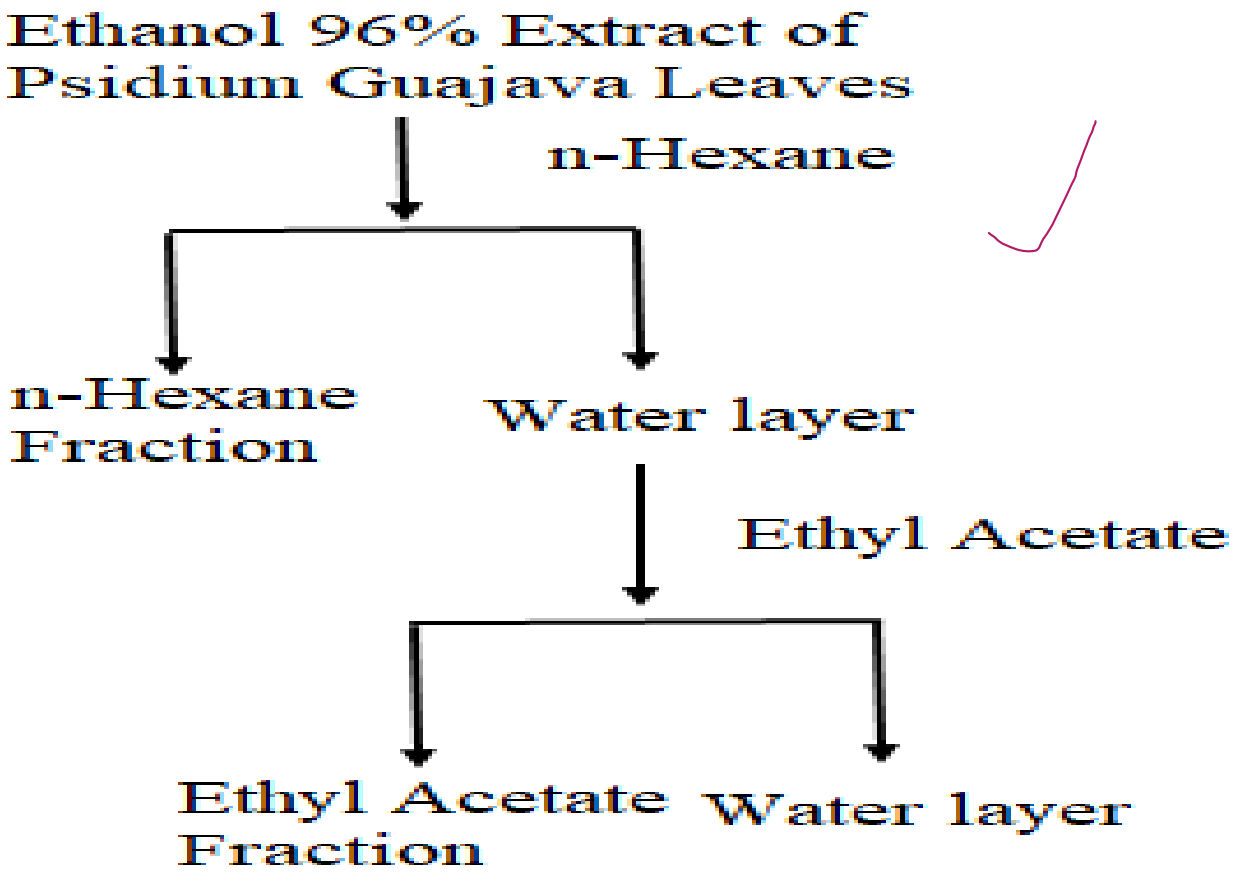

Figure 1: Fractionational processing of ethanol 96% Extract of Guajava Psidium L. Leaves

Determination of Phenol Total Conten

The total phenol was determined using Folin Ciocalteu method. Gallic acid standards 10 mg dissolved in 100 mL aquadest were made in various concentrations; 30, 40, 50, 60, 70 and 80 ug/ml.

Then, 0.3 mL solution of gallic acid standard was mixed with 1.5 mL solution of Folin Ciocalteu reagent (1:10, v/v) and the solution was stirred until homogeneous. After 5 minutes, 1.2 mL of 7.5% sodium carbonate was added and waited for 71 minutes.

Then the solution was measured at a wavelength of 743 nm using UV-Vis spectrophotometer. 100.0 mg sample of ethanol extract of Psidium guajava L. leaf was dissolved with distilled water to a volume of 25.0 ml. Then, the identification of total phenol content of ethanol extract of Psidium guajava L. leaf was carried out in the same way with procedure of the solution of Gallic acid standard solution.

Scavenging of Free Radical of DPPH

Determination of the free radical scavenging activity of Psidium guajava leaf was done by DPPH method. 1.0 ml sample was added 1.0 mL solution of DPPH 1 mM then shaken and waited for 30 min. The color of the DPPH solution changed from purple to yellow which indicated the free radicals scavenging activity of DPPH. Furthermore, absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The equation of DPPH radical scavenging activity is as:

$$IC_{50}(\%) = \frac{(\text{absorbance of control} - \text{absorbance of sample}) \times 100\%}{\text{absorbance of control}}$$

Antibacterial Activity of Staphylococcus Aureus

The antibacterial activity was tested by agar well diffusion method. 8 mg/ml of ethanol 96% extract of Psidium guajava leaves and its fraction put on 5 mm of hole on mueller Hilton Agar plate (MHA). Zone inhibition was observed after 24 hours on incubation condition at 37^{0}C.

Result and Discussion

Yield of the Extract of Guajava psidium L. and its Fractions

The extraction process of herbal plants is very important because it will determine the amount of active compound that will be extracted from herbal plants source. Therefore, the extraction process needs to be optimized to obtain the active compound. So, this research will take up the effect of various solvent in the extraction and fractionation process of active compound from Psidium guajava leaves. The yield result showed on Table I.

Table 1: Yield of Guajava Leaf Extract and Its Fractions (%)

Samples	Yield (%)
Extract 96%	15.9495%
N Hexane Fraction	54, 704 %
Ethyl Acetate Fraction	20, 378 %
Fraction of Water	79, 620 %

The yield each fractions were different, it indicated that the differences of solvent has different effect and capability in the extraction or fractionation process. The lowest yield in this field was ethyl acetate fraction of Psidium guajava leaves. The most field was n hexane fraction of Psidium guajava leaves.

Screening Phytochemical of Psidium Guajava L

Extract ethanol of Psidium guajava L and its fraction was determined the phytochemical compounds. First, the fraction of extract etanol of Psidium guajava leaves was extracted like figure 1. Generally, the result of screening of phytochemical was showed in the Table 2. The research from [8] also reported that alcoholic extract of guava leaves has several compounds such as flavonoids, alkaloids, glycosides, tannins, steroids, vitamins and carbohydrates, but do not contain saponins. No detection of saponins and tannins was also found in the methanol extract of guava leaves [9]. While the research showed in the ethanol extract of

guava leaves contains saponins. This difference showed that any difference of ability solution to dissolve the active compound in guava leaves, because the polarity of the solvent used is different. Whereas, ethyl acetate fraction from the ethanol extract of guava leaves has the same active compound.

Ethanol extract of guava leaves and ethyl acetate fraction resulted the phytochemical screening with various types of active compounds such as phenolic compounds, saponins, alkaloids and even flavonoids. This is consistent with research from [10] which states that ethanol can extract compounds on guava leaves with many active compounds.

Table 2: Phytochemical Compound of Psidium Guajava L

Qualitative Parameters	Extract of Guava			
	Ethanol 96%	N Hexane fraction	Ethyl Acetate Fraction	Water Fraction
Phenol	+	-	++	+
Flavonoids	+	+	+	-
Saponin	++	-	++	+
Alkaloids (Dandendrof Test)	++	++	++	+
Alkaloids (Mayer Test)	+	++	++	-

+: Quantity of active compound

Total Phenolic Content and Its Antioxidant Activity of DPPH

The research indicated that each ethanol extract of guava leaves and their fractions have different antioxidant activities. The results of antioxidant activity against DPPH were seen from IC_{50} values. The IC_{50} value of the ethanol extract of guava leaves and ethyl acetate fractions had adjacent values of 10.83 ± 0.58 ug/mL and 9.07 ± 0.4 ug/mL (Table 3).

The antioxidant activity of ethanol extract of guava leaves and their ethyl acetate fraction have twice antioxidant activity lower than that of standard quercetin. However, this result is better than that reported by reference [6]. The antioxidant activity encourages the potential of ethanol extract of guava leaves or its ethyl acetate fraction as a potential source of herbal medicine. Reference [11] reported that guava (Psidium Guajava) has many health benefits and needs researchers to be more comprehensive in clinical trials.However, the antioxidant activity depending on the active compound in the samples. While, the active compound or secondary metabolites in guajava leaves also depends on composition of soil nutrient where the guajava tree was planted.

The highest phenol total content was found in water solvent that has value 26%. Phenol has high polarity, so it can find highest in the water solvent. This research showed that ethanol extracts 96%, n hexane fraction, ethyl acetate fraction, and water residu of guajava leaves has free radicals scavenging activity of DPPH.

This is a potential for guajava leaves to be herbal products. Phenol as active compound has unproportional correlation to the antioxidant activity of guajava leaves (Table 3). In this research showed that the flavonoid content as influential coumpound in the activity antioxidant.

Table 3: The Phenolic Total Content and Free Radical Scavenging Activity (IC_{50}) of Guava Leaf

Samples	Phenol Total Content (%±sd)	Antioxidant Activity of DPPH (IC_{50}, ug/mL±sd)
Ethanol 96 %	15.38±0.97	10.83±0.58
N Hexane Fraction	7.80±0.22	38.63±2.32
Ethyl Acetate Fraction	18.64±0.56	9.07±0.4
Water residu	26.00±1.17	13.45±0.91
Quercetin	Na	4.23±0.21

Na: not Analyzed, four replicates

Antibacterial Activity of Staphylococcus Aureus

Antibacterial is one of parameter to show up that a herb plant has activities which it encourage the application. Ethanol 96% Extract of Psidium guajava L. Leaves and its fraction was tested the antibateri activities especially Staphylococcus Aureus. Result of this research showed on Figure 1.

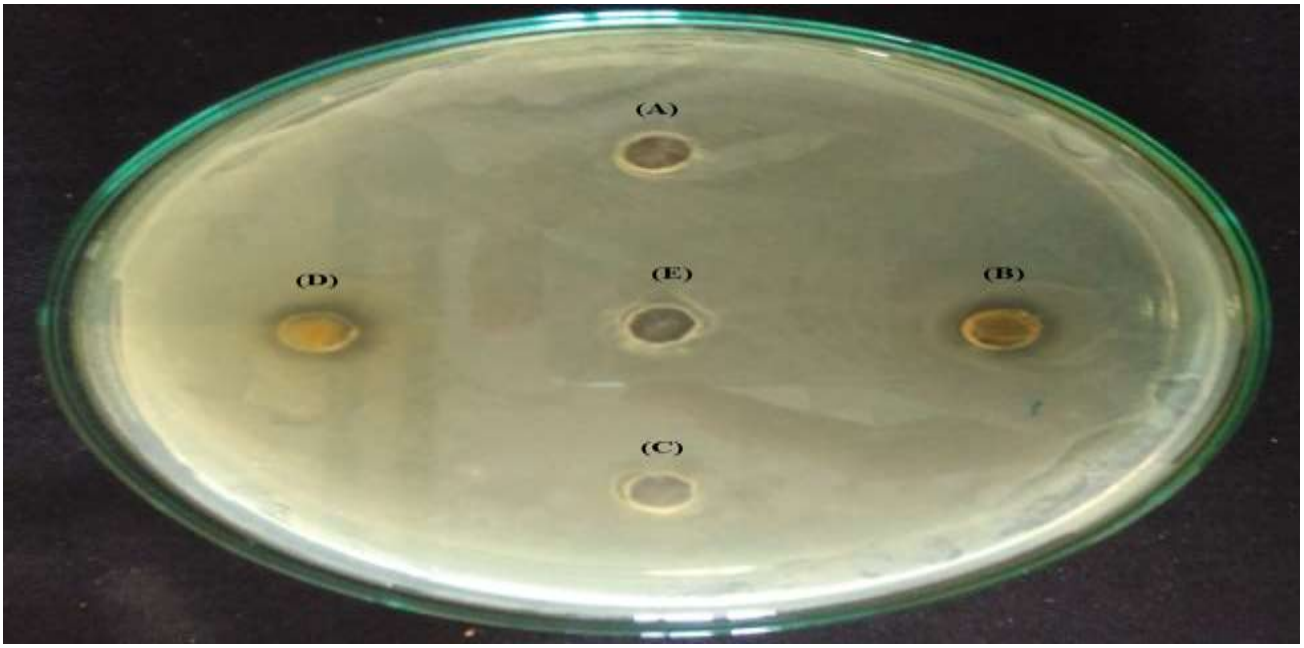

Figure 2: Antibacterial Activity of Staphylococcus Aureus from Ethanolic Extract and its Fraction of Psidium Guajava L leaves : (A) Water Fractions, (B) Ethyl Acetate Fraction, (C) n Hexane Fraction, (D) Extract Ethanol 96%, and (E) Solvent Controlled

Base on research from [12] showed that ethanol extract of Psidium Guajava leaves can inhibit the growth of some pathogens bacterial such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Salmonella typhi. The ethanol extract of Psidium guajava leaves has minimum inhibition concentration (MIC) and minimum bacterial concentration (MBC) at extract concentration of 25 mg/ml [13].

The research from [14] also reported that ethanol extract of Psidium guajava L leaves with concentration of 5% and 20% have antibaterial activity are 15.34 mm and 21.34 mm respectively. This research conducted that the ethanol 96% extract has potential as antibacteri which it has diameter inhibition of 6.25±0.43 mm. The scheme of antibacterial activities potential of Guajava extracts are ethanol 96% extract = ethyl acetate fraction > n-hexane fraction = water fraction (Tabel 4).

Table 4: Diameter Inhibition of Psidium Guajava Leaves on Staphylococcus Aureus

Samples	Diameter inhibition (mm±Sd)*
Ethanol 96%	6.25±**0.43**[a]
N Hexane Fraction	1.25±**0.43**[b]
Ethyl Acetate Fraction	5.83±**0.72**[a]
Water residu	1.17±**0.29**[b]
Solvent (Aquades)	0

*Tree replicates,
[a]Statistics result (sig: 0.333)
[b]Statistics result (sig: 0.842)

Conclusion

The ethyl acetate is a good solvent in the fractionation process of Guajava leaves because it has highest antioxidant activity with IC_{50} is 9.07±0.4 ug/mL. It is also support by the result of screening phytochemical that ethyl acetate fraction was contain mostly completed of chemical substances then other solvent such as ethanol extract 96%, n-hexane fraction, and water residu.

Water is a good solvent to extract phenol total content because phenol can be dissolved in the water and the higest phenol total content was found 26.00% in the water residu. Other result showed that the bacterial activity of ethanol 96% extract and ethyl acetate fraction are 6.25±0.43 mm and 5.83±0.72 mm, respectively.

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