INTERNATIONAL CONFERENCE ON PHARMACEUTICAL RESEARCH AND PRACTICE
ISBN: 978-979-98417-5-9 | Universitas Islam Indonesia

Antioxidant Test, Phenolic And Flavonoid Content Ethanol Extract And Ethyl Acetate Fraction Of Purple Passion Fruit Peel (*Passiflora edulis* Sims.)

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Abstract. Purple passion fruit (*Passiflora edulis* Sims.), Passifloraceae family. In order to conduct a research for the utilization of purple passion fruit peel as an eye remedy, antioxidant test, phenolic and flavonoid content to ethanol extract and ethyl acetate fraction of passion fruit (*Passiflora edulis* Sims.) peel should be examined. The peel of the fruits is macerated with 96% ethanol, then fractionated using ethyl acetate. Ethanol extract and ethyl acetate fraction were tested for their antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl. To determine the phenolic content and flavonoid content were calculated as Gallic Acid Equivalent (GAE) with Folin Ciocalteu and Flavonoid as Quercetin Equivalent (QE) with $AlCl_3$ by using colorimetric method. The results showed that both ethanolic axtract and ethyl acetate fraction were had antioxidant activity and medium phenolic content and flavonoid content.

Keywords: *Passiflora edulis* Sims., antioxidant, phenolic, flavonoid, colorimetric.

1. Introduction

Many diseases such as cancer, heart disease, arthritis, diabetes, liver, and degenerative diseases are increasingly common among people in Indonesia. One of them can be caused by antioxidants in the body unable to neutralize the increase in the concentration of free radicals. Free radicals are molecules that in the outer orbit have one or more unpaired electrons, are very labile and very reactive so they can cause damage to cell components such as DNA, lipids, proteins and carbohydrates. This damage can cause various biological disorders such as atherosclerosis, cancer, diabetes and other degenerative diseases [1].

Flavonoids are one of the largest and most natural phenolic antioxidant compounds found in all plants, so it can be ascertained that there are flavonoids in each study of plant extracts. Several medicinal plants containing flavonoids have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, allergic and anticancer activities [2]. Flavonoid compounds are polyphenol compounds with a nucleus consisting of 15 carbon atoms, composed of two rings of benzene groups connected to one by a linear chain consisting of 3 carbon atoms. Flavonoids are generally found in plants bound to sugar as glycosides [3].

Flavonoid compounds have the potential to provide high antioxidant activity [1]. According to previous studies the inhibitory concentration 50 (IC_{50}) value of ethyl acetate fraction from purple passion fruit (*Passiflora edulis* Sims.) peel was 13.82 µg / ml. The strong antioxidant activity ethyl acetate fraction from purple passion fruit (*Passiflora edulis* Sims.) peel need to determine the phenolic and flavonoids, because these two groups of compounds support the value of antioxidant activity. Purple ma passion fruit (*Passiflora edulis* Sims.) peel has gone through several previous

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studies, as antiinflammatory [4], and preliminary research on toxicity tests [4, 5].

In this study the antioxidant activity will be tested from the ethanolic extract and ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel, using the 1,1-diphenyl-2-picrylhydrazyl method [6]. This study also determine the phenolic and flavonoid content the ethanolic extract and ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel, using the Folin Ciocalteu method [7].

2. Methodology

2.1 Materials

The materials used in this research were ethanolic extract and ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel (Ginting, et, al., 2016), gallic acid (Sigma Aldrich), ascorbic acid (Sigma Aldrich), 1,1-diphenyl-2-picrylhydrazyl (Sigma Aldrich), Folin Ciocalteu (Sigma Aldrich), $AlCl_3$ (Merck), Na_2CO_3 (Merck), CH_3COOK (Merck), methanol (Merck), ethanol (Merck), Water (Brataco), and other reagent with pro analysis grade.

2.2 Tools

The tools used in this research were rough balance (Ohaus), analytical balance (Sartorius), electricity balance (Vibra), rotary evaporator (Stuart), UV-Vis spectrophotometer (Shimadzu), glassware (Iwaki).

2.3 Test of Antioxidant Activity by Colorimetric Method

Determination of Maximum Wavelength (λ_{max}) and Operating Time of 1,1-Diphenyl-2-Picrylhydrazyl. The 1,1-diphenyl-2-picrylhydrazyl was made at concentrations of 40 µg/mL and measured the absorbance at 200 nm to 800 nm to abtained the maximum wavelength [6]. The absorbance of the solution was measured every minute for 60 minutes to obtained the operating time.

2.4 Determination of Antioxidant Activity of Ascorbic Acid

The ascorbic acid (50 mg) was dissolved in methanol (50 mL) to resulted sample mixture with concentration 1000 µg/mL. Pipetted 1.25 mL, 2.50 mL, 5.00 mL, 7.50 mL, and 10.00 mL, added 5 mL of 1,1-diphenyl-2-picrylhydrazyl solution 0.5 mM (200 µg/mL), added with methanol to the marked line, then allowed to stand until the operating time. Each of the mixture was measured the absorbance at maximum wavelength.

2.5 Determination of Antioxidant Activity of Samples

Each of the ethanolic extract and the ethyl acetate fraction (50 mg) was dissolved in methanol (50 mL) to resulted sample mixture with concentration 1000 µg/mL. Pipetted 1.25 mL, 2.50 mL, 5.00 mL, 7.50 mL, and 10.00 mL, added 5 mL of 1,1-diphenyl-2-picrylhydrazyl solution 0.5 mM (200 µg/mL), added with methanol to the marked line, then allowed to stand until the operating time. Each of the mixture was measured the absorbance at maximum wavelength.

2.6 Calculation of Scavenging Concentration 50 (SC_{50})

The ability of the antioxidant activity of the samples and ascorbic acid was measured as a decrease in the absorbance of 1,1-diphenyl-2-picrylhydrazyl (discoloration) due to the addition of samples or ascorbic acid solution.

$$\text{Scaveging Percentage} = \frac{\text{Absorbance Without Addition - Absorbance With Addition}}{\text{Arbsorbansi Without Addition}} \times 100\% \quad (1)$$

The results of scavenging percentage obtained were used for regression equation calculation with sample concentration (µg/mL) as abscissa (X axis) and scavenging percentage as the ordinate (Y axis). So that a regression line is obtained which can then be calculated the ability of the test material as an antioxidant by calculating scavenging concentration 50 (SC_{50}) by substituted 50 to the Y value.

2.7 Determination of Total Flavonoid by Colorimetric Method

Determination of Maximum Wavelength (λ_{max}) and Calibration Curve of Quercetin with $AlCl_3$

Quercetin (25 mg) was dissolved in methanol (1000 mL) to resulted quercetin solution with concentration 25 µg/mL, then made a series of standard solutions with concentration 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL and 5 µg/mL. Each concentration was pipetted 0.5 mL, added 1.5 mL ethanol, added 0.1 mL of $AlCl_3$ 10%, added 0.1 mL of CH_3COOK 1 M, and added 2.8 ml of distilled water, then incubated the mixture for 30 minutes at 25°C. The mixture with concentration 3 µg/mL was measured the absorbance at 200 nm to 800 nm to abtained the maximum wavelength. The series concentration was

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measured the absorbance at maximum wavelength to obtained the calibration curve and the regression equation.

2.8 Determination of Total Flavonoid of Samples

Each of the ethanolic extract and the ethyl acetate fraction (50 mg) was dissolved in methanol (50 mL) to resulted sample mixture with concentration 1000 µg/mL. Pipetted 8 mL of each solution added with methanol to 25 ml (320 ppm concentration). Each extract was pipetted 0.5 mL, added 1.5 mL ethanol, added 0.1 mL of $AlCl_3$ 10%, added 0.1 mL of CH_3COOK 1 M, and added 2.8 ml of distilled water, then incubated the mixture for 30 minutes at 25°C. Each of the mixture was measured the absorbance at maximum wavelength. Flavonoid concentrations were calculated from the calibration curve and the regression equation, results were plot and expressed in amount of milligrams of quercetin equality in per of sample [8].

2.9 Determination of Total Phenolic by Colorimetric Method

Determination of Maximum Wavelength (λ_{max}) and Calibration Curve of Gallic Acid. Gallic Acid (100 mg) was dissolved in ethanol (100 mL) to resulted gallic acid solution with concentration 1000 µg/mL, then made a series of standard solutions with concentration 200 µg/mL, 225 µg/mL, 250 µg/mL, 275 µg/mL and 300 µg/mL. Each concentration was pipetted 0.2 mL, added 15.8 mL water, added 1.0 mL of Folin Ciocalteu reagent, shaken until homogeneously, allowed to stand for 8 minutes, added 3 mL of Na_2CO_3 solution, shaken homogeneously, then allowed to stand for 2 hours. The mixture with concentration 250 µg/mL was measured the absorbance at 200 nm to 800 nm to abtained the maximum wavelength. The series concentration was measured the absorbance at maximum wavelength to obtained the calibration curve and the regression equation.

2.10 Determination of Total Phenolic of Samples

Each of the ethanolic extract and the ethyl acetate fraction (50 mg) was dissolved in ethanol (50 mL) to resulted sample mixture with concentration 1000 µg/mL. Pipetted 8 mL of each solution added with methanol to 25 ml (320 ppm concentration). Each extract was pipetted 0.2 mL, added 15.8 mL water, added 1.0 mL of Folin Ciocalteu reagent, shaken until homogeneously, allowed to stand for 8 minutes, added 3 mL of Na_2CO_3 solution, shaken homogeneously, then allowed to stand for 2 hours. Each of the mixture was measured the absorbance at maximum wavelength. Phenolic concentrations were calculated from the calibration curve and the regression equation, results were plot and expressed in amount of milligrams of gallic acid equality per g of sample.

3. Results and discussions

The 1,1-diphenyl-2-picrylhydrazyl are widely used as a radical model for antioxidant testing. Phenolic compounds in plants can trapping the radicals. The mechanism of phenolic compounds in trapping the radicals is through proton donations [9]. The presence of electron transfer and the transfer of hydrogen atoms between antioxidants and radical will reduce the radicals. In this research the reduction of 1,1-diphenyl-2-picrylhydrazyl will cause the discoloration from purple to yellow [10]. In the purple 1,1-diphenyl-2-picrylhydrazyl radical, this color will become light yellow after receiving protons. Thus the principle of measuring activity through a decrease in absorbance at a length of 515 nm [9[.

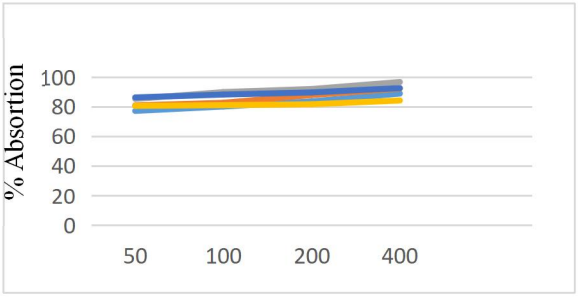

Fig 1. Relationship of absorbance of DPPH to increase concentration of test solution in analyzing antioxidant activity

Flavonoid and phenolics levels in plants were vary and depended to part, maturity, and environmental factors such as temperature, nutrition, water availability and CO_2 levels in the atmosphere [11]. In the measurement of total flavonoid compounds, the sample solution was added with $AlCl_3$ which can form a complex, so that a wavelength shift towards visible range which is marked with a solution produces a more yellow color [12]. The addition of CH_3COOK aims to maintain the wavelength in the visible area [13]. The incubation treatment for 1 hour before the measurement was intended to make the reaction run perfectly, so that the color intensity produced was maximized [8]. The results of this study obtained total flavonoid content in ethanolic extract and

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ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel can be seen in Table 1.

Table 1. Total flavonoid content in ethanolic extract and ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel

Sample	Total Flavonoid Content (mg Quercetin Equivalent per g Sample)
Ethanolic Extract	11.93
Ethyl Acetate Fraction	13.95

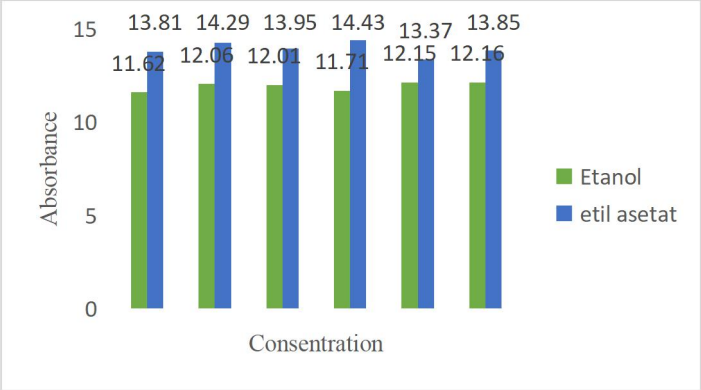

Fig 2: Graphic of the Total Flavonoid Content

In this study, total phenolic content were obtained by Folin Ciocalteu reagent with gallic acid as the standard. Although the exact mechanism of the reaction that occurs in the Folin-Ciocalteu reagent is unknown, but basically it is the reduction of the phosphomolybdothungstate compound into a blue heteropolymolybdenum [14]. Galic acid is a natural phenol compound derived from hydroxybenzoic acid. Gallic acid is reacted with Folin Ciocalteu in an alkaline atmosphere which produces a yellow color which indicates positive or contains phenol. During the reaction, hydroxyl groups in phenolic compounds react with Folin Ciocalteau reagents [15]. The results of this study obtained total phenolic content in ethanolic extract and ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel can be seen in Table 2 and Figure 2.

Table 2. Total phenolic content in ethanolic extract and ethyl acetate fraction of purple passion fruit (*Passiflora edulis* Sims.) peel

Sample	Total Phenolic Content (mg Gallic Acid Equivalent per g Sample)
Ethanolic Extract	136.97
Ethyl Acetate Fraction	150.46

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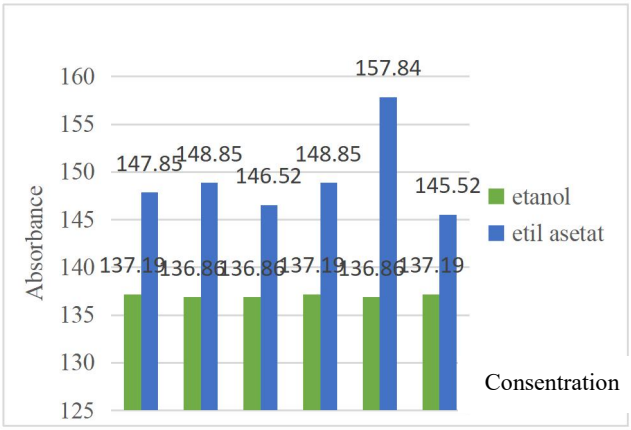

Fig 3. Grafic of the total Phenolic content

Phenolic compounds are reacted in an alkaline atmosphere to allow proton dissociation to become phenolic ion. The base solution added in determining the levels of total phenolic compounds is Na_2CO_3 solution [15].

4. Conclusions

The very strong antioxidant activity of ethyl acetate fraction from purple passion fruit (*Passiflora edulis* Sims.) peel ethanolic extract was observed with SC_{50} value 13.20 µg/mL. The phenolic content of ethanolic extract was 136.97 mg Gallic acid Equivalent per g extract and flavonoids content of ethanolic extract was 11.93 mg Quercetin Euivalent per g extract. The phenolic content of ethyl acetate fraction was 150.46 mg Gallic acid Equivalent per g extract and flavonoids content of ethyl acetate fraction was 13.95 mg Quercetin Euivalent per g extract.

5. Acknowledgement

The authors acknowledge the financial support by Research Institutions, University of Sumatera Utara, in accordance with the Contract Research TALENTA University of Sumatera Utara, Fiscal Year 2018.

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