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Testing Antioxidant Activity of Plumeria Alba and Plumeria Rubra Ethanolic Extracts Using DPPH and Frap Methods and Determining Their Total Flavonoid and Phenolic Levels

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Abstract

Plumeria sp. or Frangipani with local name; Kamboja plants have been reported to have antimicrobial, anticancer, and antioxidant activities spread in every part of the plant. Previous studies that have been carried out only reported antioxidant activity in certain parts with certain methods. This study compared the most potent antioxidant activity in several parts of the plant with different methods with statistically significantly different results. This study aims to determine the antioxidant activity of *Plumeria alba* L. and *Plumeria rubra* L. by DPPH and FRAP methods and to determine the total flavonoid and phenolic levels. The methods used are two different methods, namely the DPPH and FRAP methods. Antioxidant activity test using the DPPH method showed that the highest antioxidant activity was found in P. rubra flowers with an IC50 value of 150, 20 ppm while in the FRAP method the highest activity was in *P. rubra* leaves with an antioxidant activity value of 79.75 mg AAE / g extract. The highest flavonoid content was found in the sample extract of *P. rubra* leaves with 117.83 mg quercetin equivalent/g extract, while the highest total phenolic content was found in *P. rubra* flowers with 108.85 mg gallic acid equivalent/g extract. The results showed that the highest antioxidant activity using the method DPPH correlated with phenolic content while the FRAP method correlated with flavonoid content. Antioxidant activity in *P. rubra* leaves comes from flavonoid compounds while in *P. rubra* flowers come from phenolic compounds. So that each plant sample can have a source of antioxidant activity from different compounds.

Keywords: *Plumeria alba* L., Plumeria rubra L., DPPH, FRAP.

INTRODUCTION

Currently, many plants are used and researched as a source of natural antioxidants. Antioxidants have an important role in overcoming the effects of free radicals that cause carcinogenic diseases such as diabetes mellitus, hypertension, coronary heart disease, cancer, signs of aging, and arteriosclerosis. Sources of natural antioxidants obtained from plants generally contain flavonoid compounds and phenolic compounds that are scattered in plant parts (Anwar and Triyasmono, 2016). *Plumeria sp*. or frangipani plant is a flowering plant originating from Central America which then spread throughout the

world. Until now, frangipani plants are often found in Indonesia. *Plumeria sp*. has many types, including *Plumeria alba* L. or frangipani with white flowers and *Plumeria rubra* L. or frangipani with red flowers. Previous studies that have been carried out only reported antioxidant activity in certain parts with certain methods. This study compared the most potent antioxidant activity in several parts of the plant with different methods with statistically significantly different results.

Plumeria sp. was reported to have antimicrobial activity (Sibi et al., 2012), anticancer (Radha and Sivakumar, 2009), and antioxidant (Merina, 2010). Devprakash et al. (2012) stated that the extract of Plumeria sp. It is known to contain alkaloids, glycosides, terpenoid reducing sugars, saponins, tannins, carbonyls, flavonoids, and steroids. Research by Rahman et al. (2014) reported that the flower extract of *Plumeria alba* L. is a potential source of antioxidants and has cytotoxic activity, and *Plumeria rubra* L. also has antioxidant and hypolipidemic activity from the presence of flavone glycoside compounds (Choudhary et al., 2014). One of the reported activities of *Plumeria sp*. is the antioxidant activity where antioxidants are compounds that inhibit free radicals (Mandal et al, 2009).

Derivatives of phenolic compounds are secondary metabolites produced by plants. One of the largest groups of phenolic compounds is the flavonoid group. Phenolic compounds are natural compounds that have the ability as biologically active compounds, one of which is as an antioxidant which is considered capable of preventing and treating degenerative diseases (Apsari and Susanti, 2011). Research conducted by Aldo (2012) regarding antioxidant activity tests and determination of total phenolic levels revealed that there was a correlation between strong antioxidant sources and the content of total phenolic compounds. Ghasemzadeh and Ghasemzadeh (2011) also revealed that phenol and flavonoid compounds have a linear contribution to antioxidant activity and the higher the level, the better the antioxidant activity.

This paper is the result of research on the Antioxidant Activity Test of *Plumeria alba* L. and *Plumeria rubra* L. Ethanol Extracts with DPPH and FRAP Methods and Determination of Total Flavonoid and Phenolic Levels. The modification of the research to the previous research is *Plumeria sp*. The research used focuses on the ethanol extract of the flower, leaf, and stem bark of *Plumeria alba* L. and *Plumeria rubra* L. with two different methods, namely DPPH and FRAP, and the determination of flavonoid and total phenolic levels.

METHODS

1. Tools and Materials

The tools used in this research are glassware (Pyrex), volume pipette (Iwaki), measuring pipette, analytical balance (Ohaus), micropipette (Socorex), macro pipette (Socorex), rotary evaporator (Heidolph), water bath (Memmerth), tube reaction, maceration container, vacuum Buchner, filter paper, stirring rod, porcelain dish, aluminum foil, blue tips, ependolph, stopwatch, pH-meter, UV-Vis spectrophotometer (UV Mini SHIMADZU), 1 mL cuvette (Hellma), sonicator (Branson), and ovens.

The materials used were flower parts, leaves, and stem bark of Plumeria alba L. and Plumeria rubra L. obtained from the Haji Mausoleum Surakarta, 96% technical ethanol, ethanol pa (Merck), 0.4 mM DPPH, ascorbic acid, acid oxalate, 0.2 M phosphate buffer, NaOH, KH_2PO_4, Potassium Ferricyanide ($K_3Fe(CN)_6$) 1%, TCA 10%, $FeCl_3$ 0.1%,

aquadest, CO_2-free aquadest, $AlCl_3$ 10%, sodium acetate 10%, quercetin, and 1 M potassium acetate.

2. Research Steps

2.1. Sampling

Samples of *Plumeria alba* L. and *Plumeria rubra* L. were taken in the Makam Haji area, Surakarta in August 2019.

2.2. Determination of Plant Sample

Plant samples in the form of plant parts were determined at the Biology Laboratory of the Faculty of Mathematics and Science, Sebelas Maret University, Surakarta with the results of the plant samples tested including the species *Plumeria alba* L. and *Plumeria rubra* L. The results of the determination of plant samples are attached in the appendix.

2.3. Sample Extraction

The research sample powder was macerated in 96% ethanol for 3x24 hours with a ratio of dry powder: ethanol 1:7.5 (g/mL) then the extract was concentrated with a rotary evaporator and dried in a freeze dryer.

2.4. Preliminary Test

Preliminary tests were carried out on all sample extracts, namely extracts from each flower, bark, and leaf of P. alba, as well as flowers, bark, and leaves of P. rubra using the DPPH method.

2.4.1. Preparation of Blank Solution

The blank solution was prepared using ethanol pa.

2.4.2. Creation of DPPH Control

500 L of 0.4 mM DPPH solution was added with 1 mL of ethanol pa and the absorbance was measured at a wavelength of 516 nm.

2.4.3. Measurement of Antioxidant Activity DPPH Method

The absorbance measurement of the sample is carried out by; 12.0 mg of sample extract was dissolved in 10.0 mL of ethanol pa, then 500 L was taken and 500 L of ethanol pa and 500 L of DPPH solution were added. The solution was allowed to stand in the dark for 45 minutes and then read the absorbance at a wavelength of 516 nm. The concentration of the sample used is 1200 ppm. The amount of antioxidant power is measured by the formula % free radical binding (% PRB).

2.5. Antioxidant Activity Test by DPPH Method

2.5.1. Preparation of Blank Solution

The blank solution was prepared using ethanol pa

2.5.2. Creation of DPPH Control

500 L of 0.4 mM DPPH solution was added with 1 mL of ethanol pa and the absorbance was measured at a wavelength of 516 nm.

2.5.3. Measurement of Antioxidant Activity DPPH Method

The absorbance measurement of the sample is carried out by; 12.0 mg of sample extract was dissolved in 10.0 mL of ethanol pa, then 500 L was taken and 500 L of ethanol pa and 500 L of DPPH solution were added. The solution was allowed to stand in the dark for 45 minutes and then read the absorbance at a wavelength of 516 nm. The concentration series made are 400 ppm, 240 ppm, 144 ppm, 86.4 ppm, 38.5 ppm, and 23.1 ppm. The amount of antioxidant power is expressed by the IC50 value.

2.6. Antioxidant Activity Test with FRAP Method

2.6.1. Preparation of Blank Solution

The blank solution was made with 1 mL 1% oxalate solution and 4 mL of 0.2 M phosphate buffer pH 6.6 was added.

2.6.2. Creating a Standard Curve

A stock solution of 1000 ppm was prepared with 25 mg of ascorbic acid dissolved in 1% oxalic acid solution to a volumetric flask of 25 mL. The stock solution was taken as much as 0.6; 0.7; 0.8; 0.9; and 1.0 mL and mixed with 1% to 10 mL oxalic acid solution to obtain standard solution concentrations of 60, 70, 80, 90, and 100 ppm.

2.6.3. Measurement of Antioxidant Activity by FRAP Method

A total of 5 mg of the extract was dissolved in 5 mL of ethanol pa, then 1 mL was pipetted and 1 mL of 0.2 M phosphate buffer pH 6.6 was added. 1% K3Fe(CN)6 solution was added to it and incubated at 50°C for 20 minutes. 1 mL of 10% TCA solution was added and centrifuged at 3,000 ppm for 10 minutes. The top layer was pipetted as much as 1 mL in a test tube, added 1 mL of distilled water and 0.5 mL of 0.1% FeCl3. The solution was allowed to stand for 10 minutes and then its absorbance was measured at a wavelength of 720 nm. The FRAP value is expressed in units of mg equivalent to ascorbic acid/gram extract.

2.7. Measurement of Flavonoid Level

2.7.1. Creation of a Standard Curve

5 mg of quercetin was weighed and then dissolved with 5 mL of ethanol, made 5 series of concentrations and 0.5 mL of each concentration was taken. 0.1 mL of 10% AlCl3 solution was added and mixed with 0.1 mL of 1M sodium acetate solution and 2.8 mL of distilled water and then left for 30 minutes. The absorbance was read at a wavelength of 426 nm.

2.7.2. Measurement of Sample Flavonoid Level

The 500 mg extract was weighed and dissolved in 5 mL of ethanol pa. The mixture was taken 0.5 mL and added with 1.5 mL of ethanol pa; 0.1 mL AlCl3 10%; 0.1 mL of 1M sodium acetate solution and 2.8 mL of distilled water were then left for 30 minutes. The absorbance was read at a wavelength of 426 nm.

2.8. Measurement of Total Phenolic Content

2.8.1. Preparation of Gallic Acid Standard Curve

2 mg of gallic acid was dissolved in 10 mL of ethanol pa then made up to 5 concentration series. Each concentration series was taken 500 L, added 2.5 mL of 10% Folin-Ciocalteu reagent in water and then incubated for 5 minutes in the dark. The solution was added with 2.5 mL of 7.5% NaHCO3 and incubated for 45 minutes and the absorbance was measured at a wavelength of 752 nm.

2.8.2. Measurement of Phenolic Content of Sample

5 mg of the extract was dissolved in 5 mL of ethanol pa and obtained a concentration of 1000 ppm. The solution was taken as much as 500 L and added 2.5 mL of 10% Folin-Ciocalteu reagent in water and then incubated for 5 minutes in the dark. The solution was added with 2.5 mL of 7.5% NaHCO3 then incubated for 45 minutes in the dark and the absorbance was measured at a wavelength of 752 nm.

RESULTS AND DISCUSSION

1. Sample Extraction

The extraction process aims to take the chemical compounds contained in the sample using a certain solvent. Sample extraction in this study was carried out by maceration of the sample for 3x24 hours while stirring occasionally. Maceration was chosen because this extraction method is easy to work with and the equipment used is simple. At the time of immersion, there will be contact between the filter solution and the powder so that the active substance possessed by simplicia will be dissolved into the solvent (Anwar and Triyasmono, 2016). The solvent used for the extraction was 96% technical ethanol. The ethanol solvent is used because it has polar properties where polar compounds are compounds that are soluble in water, besides that the solvent is easy to obtain and is universal. According to Hanani (2016), Flavonoid compounds generally bind to sugars to form glycosides which cause these compounds to be easily soluble in polar solvents. In addition, ethanol is a solvent that has a good ability to penetrate cell walls so that it is easier to extract secondary metabolites (Tiwari et al, 2011). In the process, the sample extract can be dissolved with ethanol so that ethanol is the right solvent. The extract obtained from the maceration was then concentrated with a rotary evaporator to obtain a thick extract. The weight of the simplicia extracted were 300.05 g, 300.03 g, and 200.01 g, respectively, while the leaves, flowers, and bark of P. rubra were 200.06 g, 100.02 g, and 300.05 g. The total extract of P. alba leaves, flowers, and bark were 15.22 g, 14.78 g, and 25, respectively.

2. Preliminary Test

Preliminary tests were carried out using the DPPH method on all sample extracts, namely P. alba flower extract, P. alba bark, P. alba leaves, P. rubra flowers, P. rubra stem bark, and P. rubra leaves intending to know the sample extracts. which one has the most potent antioxidant activity so that the next test can be continued. The preliminary test or initial screening test was chosen with the DPPH method because it is easy to do, cheap, fast, and has fairly high accuracy (Niken, 2010). Before reading the preliminary test, read the DPPH control first. The control absorbance data obtained is 1,000 with a maximum wavelength of 516 nm. Preliminary tests showed a positive reaction to the presence of antioxidant compounds. The data on the results of the preliminary test readings are in Table 1.

Table 1. Preliminary Test Results with DPPH

Sample	Absorbance	% FRB
Leaves of *P. rubra*	0.229	77.10
The bark of *P. rubra*	0.566	43.40
Flower of *P. rubra*	0.146	85.40
Leaves of *P. alba*	0.432	56,80
Flower of *P. alba*	0.238	76,20
The Bark of *P. alba*	0.485	51.50

The value of %FRB or percent of Free Radical Binding is expressed by the formula in Equation (1).

$$\% \text{ FRB} = \left| \frac{absorbance\ control - absorbance\ sample}{absorbance\ control} \right| \text{ x } 100\% \qquad (1)$$

The data in Table 1 shows the value of % FRB from the largest being *P. rubra* (85.40%). While the other % FRB values, namely *P. rubra* leaves (77.10%), *P. alba* flowers (76.20%), *P. alba* leaves (56.80%), *P. alba* bark (51.50 %), and *P. rubra* (43.40%). The three most potent sample extracts, namely the bark of *P. rubra* (85.40%), *P. rubra* leaves (77.10%), and *P. alba* flowers (76.20%) were then used for the next test. Preliminary tests carried out in this study have a drawback, namely that there is no repetition, so suggestions for further research in data collection must be repeated until a minimum of 3 data is produced. The purpose of repetition is to avoid errors in the study. Errors also occur when making blank solutions in the test using DPPH where in practice only ethanol is used. Supposedly, a blank solution was prepared using the sample extract and ethanol pa as the solvent.

Previous research conducted by Rahman et al. (2014) on methanol extracts of P. alba and P. rubra flowers, the % FRB values were 81% and 71%, where the values were not much different in this study. So that the sample extracts of *P. rubra* flowers, *P. rubra* leaves, and *P. alba* flowers can be continued with the next test, namely the antioxidant activity test using the DPPH and FRAP methods and the determination of total flavonoid and phenolic levels because they are considered to contain potential antioxidant activity.

3. Antioxidant Activity Test by DPPH Method

The DPPH method is a method that uses 2,2diphenyl-1-picrylhydrazyl as a free radical source. The principle of this method is that compounds containing antioxidant activity will react with DPPH through electron donors from antioxidant compounds to DPPH or the reaction can be seen in Figure 1. This reaction causes a decrease in the intensity of the purple color in the DPPH solution. The intensity of the purple color in the DPPH solution decreases as the sample concentration increases (Chandrasekara & Kumar, 2016). The purple to yellow color change indicates that DPPH has been reduced.

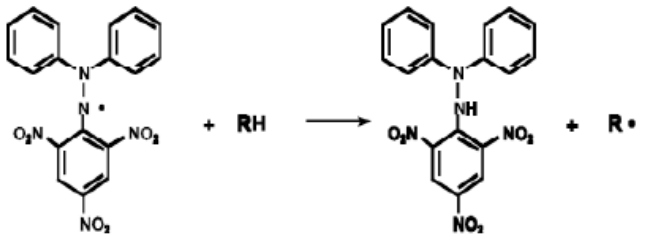

Figure 1. The reaction of Antioxidant Compounds with DPPH

The DPPH method was chosen because it can measure antioxidant activity quickly, simply, and inexpensively (Pine et al, 2015). Absorbance readings were carried out using a UV-Vis spectrophotometer with three repetitions and then calculated the IC50 value or the concentration of the test extract with the ability to scavenge free radicals of 50% obtained through the regression equation. The data obtained are as listed in Table 2 and more details are listed in the appendix.

Table 2. Antioxidant Activity Test Results with the DPPH Method

Sample	IC50 (ppm)
P. rubra leaves	203.59
P. rubra flower	150,20
P. alba flower	204.81

According to Endrini et al (2009) the more effective a compound is as a free radical scavenger if its IC50 value is getting smaller. The data in Table 2 above can be seen that the samples that have the largest antioxidant activity or the smallest IC_{50} value are *P. rubra* flower extract (150.20 ppm), then *P. rubra* leaves (203.59 ppm), and *P. rubra* flowers (204.81 ppm). The three samples were included in the category of moderate antioxidant intensity (100-250 ppm). As a positive control test, the DPPH method was carried out using vitamin E as a comparison. The IC_{50} value obtained from this positive control test is 27.97 ppm so it is included in the category of very strong antioxidant intensity (<50 ppm).

4. Antioxidant Activity Test with FRAP . Method

This study also measured antioxidant activity using the FRAP method or ferric reducing antioxidant power. The FRAP method has a different principle from the DPPH method, where the principle of this method is the reduction of the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to ferric tripyridyltriazine (Fe(II)-TPTZ) by antioxidants at low pH (Vicas et al, 2009).). Compounds (Fe(III)-TPTZ) represent oxidizing compounds in the body that can damage cells (Maryam et al, 2015). This test uses a relatively low pH, namely pH 6.6. The use of low pH can facilitate the Fe^{3+} reduction process (Syarif et al, 2015). The standard solution used is ascorbic acid. The use of ascorbic acid as a comparison is because ascorbic acid belongs to the class of secondary antioxidants that can capture free radicals and prevent chain reactions. According to research by Lung and Destiani (2018), vitamin C is more polar than vitamin A and vitamin E, so the antioxidant activity of vitamin C is stronger. The addition of TCA is intended to precipitate the potassium ferrocyanide complex. While the addition of $FeCl_3$ is intended to form a blue complex (Maryam et al, 2015). $K_3Fe(CN)_6$ can act as a provider of Fe^{3+} ions like the reaction in equation (2) below: While the addition of $FeCl_3$ is intended to form a blue complex (Maryam et al, 2015). $K_3Fe(CN)_6$ can act as a provider of Fe^{3+} ions like the reaction in equation (2) below: While the addition of $FeCl_3$ is intended to form a blue complex (Maryam et al, 2015). $K_3Fe(CN)_6$ can act as a provider of Fe^{3+} ions like the reaction in equation (2) below:

$$K_3Fe(CN)_6 \rightarrow K_4Fe(CN)_6$$

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad (2)$$

According to Maryam et al (2015), compounds that have reducing power can act as antioxidants because they can stabilize free radicals by donating electrons or hydrogen atoms so that radical compounds turn out to be more stable. $K_3Fe(CN)_6$ in this case has a reducing power where the reducing power is an indicator of the potential of an antioxidant compound. The absorbance value of the sample was read three times with a UV-Vis spectrophotometer at a wavelength of 720 nm. The FRAP value was expressed as mg equivalent of AA/g extract or mg AAE/g extract. The results of the antioxidant activity test using the FRAP method are in Table 3 and the full details are in the appendix.

Table 3. Antioxidant Activity Test Results with the FRAP Method

Sample	Average Antioxidant Activity (mg AA equivalent/g extract)
P. rubra leaves	79.75 ± 5.70
P. rubra flower	79.67 ± 4.84

P. alba flower	79.01 ± 4.09

The value of the antioxidant activity is expressed by the formula in equation (3).

$$\text{Antioxidant activity (mg AAE/g extract)} = \frac{volume\ (mL)\ x\ sample\ concentration\ (ppm)}{sample\ weight\ (mg)} \quad (3)$$

Table 3 above shows that the highest antioxidant activity value is P. rubra leaves with 79.75 ± 5.70 mg AAE/g extract, which means that in each gram of sample it is equivalent to 79.75 mg of ascorbic acid, followed by P. rubra flower with 79.67 ± 4.84 mg equivalent of AA/g extract, and the lowest was P. alba flower with 79.01 ± 4.09 mg equivalent of AA/g extract. Based on the ANOVA test, the levels of antioxidant activity in each sample extract were not significant ($p>0.05$), which means that there was no significant difference in the levels of antioxidant activity in the samples of *P. rubra* leaves, *P. rubra* flowers, and *P. alba* flowers.

This result is different from the results in the DPPH test where in the DPPH test the highest antioxidant activity was found in *P. rubra* flowers, while in the FRAP test it was found in *P. rubra* leaves. This could be due to the long period between testing with the DPPH and FRAP methods so that there are differences in sample conditions. According to Selawa, et al. (2013) antioxidant compounds are very volatile. In addition, the FRAP method has drawbacks, namely the absorbance of several phenolic compounds such as caffeic acid, ferulic acid, quercetin, and tannins that are not stable in FRAP measurements because the incubation time required is longer than the FRAP incubation time and interfering compounds can have maximum absorbance absorption in the measurement area. FRAP (Niken, 2010).

5. Determination of Flavonoid Level

Flavonoid levels and phenolic levels are two key indicators that are generally used to represent the overall antioxidant activity in the sample (Tohidi et al, 2017). Flavonoids are included in natural phenolic compounds that have the potential as antioxidants (Selawa et al, 2013). Determination of flavonoid levels in this experiment using AlCl3. AlCl3 as Lewis acid forms complex bonds with hydrophilic compounds of flavonoids. The more flavonoid content in an extract, the more visually the yellow color formed is concentrated (Niken, 2010).

Quercetin is used as a standard solution because quercetin is a flavonoid of the flavonol group which has a keto group at C-4 and a hydroxyl group at C-3 or C-5 atoms which are neighbors of flavones and flavonols (Azizah and Faramayuda, 2014). The flavonoid content was measured at a wavelength of 426 nm and the results are shown in Table 4 below in more detail in the appendix.

Table 4. Results of Determination of Flavonoid Levels

Sample	Average Flavonoid Level (mg quarcetine equivalent/g extract)
P. rubra leaves	117.83 ± 18.60
P. rubra flower	41.59 ± 2.99
P. alba flower	10.60 ± 0.62

From Table 4, it can be seen that the highest flavonoid levels were found in the sample extract of *P. rubra* leaves with 117.83 ± 18.60 mg quercetin equivalent / g

extract, which means that each gram of sample extract is equivalent to 117.83 mg quercetin. Then followed by *P. rubra* flowers with 41.59 ± 2.99 mg quercetin equivalent / g extract and the least was P. alba flowers with 10.60 ± 0.62 mg quercetin equivalents / g extract. The regression equation for flavonoid content is shown in Figure 4 which is listed in the appendix with the value (X) in the form of concentration and the value (Y) in the form of absorbance.

Based on the ANOVA test, there was a significant difference in the flavonoid levels in the *P. rubra* leaf samples ($p<0.05$) while the *P. rubra* flower and *P. alba* flower samples had no significant difference ($p>0.05$). The higher the flavonoid content, the higher the antioxidant potential. This study showed that the levels of flavonoids in *P. rubra* leaves were higher than *P. rubra* flowers, presumably because the leaves are the site of plant photosynthesis. In-plant cells that undergo photosynthesis, many phenolic compounds are found, especially flavonoids (Lukman et al, 2016). This is in line with research by Kalam et al. (2013) that P. rubra flowers have been reported to contain tannins, alkaloids, flavonoids, saponins, and terpenoids.

Research by Apak et al (2007) stated that the testing method with AlCl3 has drawbacks, namely, it can complex several groups of flavonoids such as flavones (crisine, apigenin, and luteolin) and flavonols (quercetin, myricetin, morin, and rutin) but cannot complex groups of flavonoids. flavanone and flavanonol. It can also happen in this experiment. In addition, it can be seen in Table 10 in the appendix that the concentration produced by P. rubra leaves was higher than P. rubra flowers so that the absorbance was also higher and the levels of flavonoids produced were higher. This is evidenced by the regression equation in Figure 2 ($y = 0.0062x - 0.0497$, $R^2 = 0.9943$) there is a correlation between concentration and absorbance.

6. Determination of Total Phenolic Level

Phenolic compounds as secondary metabolites in plants have the potential as antioxidants. This is due to the presence of hydroxyl groups in phenolic compounds that donate hydrogen atoms through electron transfer when reacting with radical compounds so that the oxidation process becomes inhibited (Niken, 2010). In the determination of total phenolic content, the reagent used is Folin-Ciocalteu where Folin-Ciocalteu reagent is an inorganic reagent forming complex solutions with phenolic compounds. The total phenolic content was measured using a UV-Vis spectrophotometer at a wavelength of 752 nm. The measurement results are shown in Table 5 below and more in the appendix.

Table 5. Results of Determination of Total Phenolic Levels

Sample	Average Total Phenolic Content (mg gallic acid equivalent/g extract)
P. rubra leaves	33.86 ± 3.42
P. rubra flower	108.85 ± 5.62
P. alba flower	57.79 ± 6.14

From Table 5, it can be seen that the highest total phenolic content was found in *P. rubra* flowers, namely 108.85 ± 5.62 mg gallic acid equivalent / g extract, which means that in every gram of sample extract it was equivalent to 108.85 mg gallic acid, followed by P. alba with 57.79 ± 6.14 mg gallic acid equivalent/g extract and the lowest was *P. rubra* leaves with 33.86 ± 3,42 mg gallic acid equivalent/g extract. The results of total phenolic levels in the three samples were very different. This is in line with the results of the ANOVA test where the total phenolic levels in the samples of *P. rubra* leaves, *P. rubra* flowers, and *P.*

alba flowers were significantly different ($p<0.05$). The regression equation for the total phenolic content is shown in Figure 5 which is listed in the appendix where the value (X) is the concentration and the value (Y) is the absorbance.

In previous research conducted by Rahman et al. (2014) the total phenolic content of methanolic extracts of *P. alba* and *P. rubra* flowers was 173.9 g gallic acid equivalent/mL extract and 167.3 g gallic acid equivalent/mL so that the highest concentration was *P. alba* flower. However, in this study, the total phenolic content of *P. rubra* flowers was higher than that of *P. alba*. Differences, where plants grow, can be a factor, wherein the previous study in India and this study in Indonesia. In addition, because of the difference in the solvent used. According to Zuraida et al (2017), in extracting flavonoids and phenolics, the solvent is an important factor. Chemical compounds can be extracted using ethanol more than methanol and water (Azizah and Salamah, 2013). In Figure 5 the total phenolic regression equation ($y = 0.0069x - 0.0199$, $R^2 = 0$.

There is a difference between the sequence of activity levels of flavonoids with the highest total phenolic in this study. In the determination of flavonoid content, P. rubra leaves were higher than P. rubra flowers, while in the determination of total phenolic content, it was the opposite. Thus, the high phenolic compounds do not only come from the flavonoid group because flavonoids are one of the groups of phenolic compounds. Not all flavonoids can bind to phenolic compounds, only flavonoids can bind to the benzene group and the hydroxyl group on phenolic compounds, so it is suspected that not all flavonoids have been identified. This is what causes the difference. According to Astiti (2013), there are different classes of active compounds contained in extracts of Frangipani leaves of red and white can be caused by internal factors of the plant itself such as age of the plant, the level of stress the plant, and crop growth stage that if the higher it is increasing the intensity of the compound active contained.

CONCLUSION

The conclusion in this experiment is that the strongest antioxidant activity in the DPPH method is shown by P. rubra flower extract with an IC50 value of 150.20 ppm and in the FRAP method is P. rubra leaf with 79.75 mg equivalent of AAE/g extract. The highest flavonoid content was found in the sample extract of P. rubra leaves with 117.83 mg quercetin equivalent/g extract and the highest total phenolic content was found in P. rubra flowers with 108.85 mg gallic acid equivalent/g extract. So that the highest antioxidant activity of the DPPH method correlated with phenolic content and the FRAP method correlated with flavonoid content. Antioxidant activity in P. rubra leaves comes from flavonoid compounds while in P. rubra flowers come from phenolic compounds. Each plant sample can have different sources of antioxidant activity.

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