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Article

Antioxidant Activity Test of Combination Extract from Roselle Petals (*Hibiscus sabdariffa* L.) and Calamansi (*Citrus microcarpa* Bunge.) Essential Oil Using DPPH Method

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Abstract: Roselle petals and calamansi essential oil were known contain compounds such as anthocyanins and limonen, which have potentialy as antioxidants to capture free radicals. This study was aimed to determine antioxidant activity of combination extract from roselle petals and calamansi peel essential oil. Rosella flower extract was obtained using the maceration method with 96% ethanol as the solvent and calamansi essential oil was obtained using distillation method. The antioxidant activity of combination extract was tested using DPPH method with UV-Vis spectrophotometer in wavelength of 517.50 nm. The results showed that the combination extract from roselle petals and calamansi essential oil in 1:1 and 3:1 ratio were exhibited very strong antioxidant activity, with IC_{50} values of 22.48μg/mL and 18.88 μg/mL, respectively. Meanwhile, in 1:3 ratio of combination extrcat was exhibited strong antioxidant activity with an IC_{50} value of 54,65 μg/mL. Research shows that a combination of extracts with a higher concentration of rosella extract provides better antioxidant results.

Keywords: Antioxidant, Combination-Extract, Roselle, Calamansi, DPPH

1. Introduction

Nowadays, with the development of technology and science, there has been a change in people's lifestyles. Consumption habits *fast* food *food* , consuming food with nutrition bad, less movement And exercising, not enough Rest, stay up late, consume cigarette and alcoholic beverages are some examples of unhealthy changes in people's lifestyles. style life the become reason increasing case Degenerative diseases. Degenerative diseases are diseases that affect quality of life, can reduce a person's productivity, and are chronic [1].

One of the causes of degenerative diseases is oxidative stress due to environmental influences. Oxidative stress is a condition where free radicals are out of balance with antioxidants. Oxidative stress is a key factor in the development of diabetes, cancer, cardiovascular disease, and neurodegenerative diseases. Long-term exposure to excessive pro-oxidants can trigger damage structure DNA mitochondria and interfere with function enzyme and cellular components, resulting in aberrant gene expression. Modern lifestyle, Which covers consumption food processed, exposure various material chemistry, and lack of sport, is a number of example stress oxidative [2].

Free radicals are molecules that react very easily. (reactive) and An antioxidant is a substance or molecular compound that can inhibit free radicals. To prevent and reduce oxidative damage, the human body has antioxidant defense mechanisms. These defense

mechanisms can include free radical scavenging, metal chelation, and/or enzymatic neutralization of free radicals [3].

One of the main sources of natural antioxidants is phenolic compounds. Phenolic compounds can be found in almost all parts of plants, such as leaf, fruit, stems, bark, roots, and seeds [4].Rosella flower petals and calamansi orange peel are two plant parts that have been studied to contain compounds with antioxidant potential. Research by Amriani and Tuahatu (2022) shows that rosella flower petals contain anthocyanins as Very strong potential antioxidant with an IC_{50} value reaching 43μg/mL ($IC_{50} < 50$μg/mL) [5]. Meanwhile, research by Septiani *et al.* , (2024) showed that the essential oil of calamansi orange peel has antioxidant activity with strong potential with an IC 50 value is 50.31 μg/mL [6] .

Several recent studies on antioxidant potential have often involved a combination of two sources of antioxidants, some types of antioxidants have the potential to increase the effectiveness of antioxidant activity when it combined [7] . In a study by Rudiana et al. (2020), antioxidant activity was tested on a combination of ethanol extracts of bay leaves (*Syzygium polyanthum*) and moringa leaves (*Moringa oleifera*). It was found that the combination of the two extracts increased antioxidant activity. The IC50 value was bay leaf extract and leaf extract single moringa each is 31.14 μg/mL and 71.27 μg/mL , while the value IC 50 combination of the two extracts comparison 2:1 is of 28.55 μg/mL. This means that the combination causes an increase in antioxidant activity [8] .

This study tested the antioxidant activity of a combination of ethanol extract of roselle petals and essential oil of calamansi orange peel using the DPPH method. This research is expected to provide a new reference for optimizing antioxidant strength by combining these sources.

2. Materials and Methods

2.1. Materials

The tools used in this study were analytical scales (*Lab Tech®)* , *rotary evaporator (Emeltron*), UV-Vis Spectrophotometer *(Spektro Uv-Vis Double Beam PC 8 Scanning Auto Cell Uvd-3000*), Votex Mixer (B-ONE), Cuvettes *(HELMA*), laboratory glassware *(Pyrex®),* blender, micropipette.

The materials used in this study include Rosella flowers (*Hibiscus sabdariffa* L.) and calamansi orange fruit (*Citrus macrocarpa* Bunge) obtained in Bengkulu City. The plant samples have been verified for the correctness of the plant species by the Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Bengkulu with document number 123.b/UN30.12/BIO/TA.00.03/2025 . Other materials used include distilled water (*Purelizer®*), 96% ethanol solvent, vitamin C (*Nitra Kimia®*) and DPPH radicals *(2,2-diphenyl-1-picrylhydrazyl) (Sigma-Aldrich®).*

2.2. *Methods*

a. Ethanol Extract of Flowers Rosella

The rosella flower petals were separated and dried to obtain the simple drug. A total of 55g of dried rosella flower petal powder was extracted with method maceration using 550 mL of solvent ethanol 96% during 3 day with 6 O'clock very stirring . The maceration results are filtered use paper *Whatman* filter grade 42 (90 mm) to separate the residue and filtrate. The residue from the first maceration was then remacerated with 96% ethanol for 1 day. The filtrate from the maceration and remaceration were combined for further solvent evaporation. using *a rotary evaporator* at a temperature of 40°C pressure 20 Psi with a spin 150 rpm [9] .

b. Preparation oil citrus peel essential oil Kalamansi

A total of 1.5 kg of calamansi orange peel was ground using a blender with a sample to distilled water ratio of 1:1. Essential oil was obtained using the distillation method. The prepared sample was placed in a steam distillation still at 100°C for 4 hours. Distillation was stopped if the volume of essential oil in the bottle did not increase within 30 to 45 minutes. The distillation product, a mixture of water and essential oil, was separated using a funnel. separator [6].

c. Antioxidant Activity Test of Extract Combination

- **Preparation of stock solution**

Solution parent DPPH made with weigh 4 mg powder DPPH was then put into a 100 mL measuring flask and dissolved with ethanol until it reached a final volume of 100 mL (concentration 4 μg/mL) [6].

- **Preparation of Blank Solution**

A total of 2 mL of DPPH 40 ppm and 2 mL of 96% ethanol were mixed in a test tube, homogenized by shaking, then incubated for about 30 minutes [6].

- **Preparation of Vitamin C Standard Solution**

The stock solution of vitamin C with a concentration of 100 ppm is made by weighing 10 mg of vitamin C and dissolving it in 100 mL of distilled water, then shaking it until homogeneous. The vitamin C solution with a concentration of 100 ppm is made by weighing 10 mg of vitamin C and dissolving it in 100 mL of distilled water, then shaking it until homogeneous. 2, 4, 6, 8, and 10 ppm. 1 mL of each solution was taken by adding 0.1 mM DPPH solution. The solution was then incubated at room temperature and its absorbance was measured. on long wave optimum use spectrophotometer UV-Vis [6].

- **Determination Maximum Wavelength**

After process incubation for 30 minutes in dark room, absorbance The blank solution was measured using a UV-Vis spectrophotometer at a wavelength between 470 and 550 nm [6].

- **Preparation sample solution**

A number of extract thick petals flower rosella And oil essential oils Kalamansi was weighed in ratios of 1:1, 1:3, and 3:1, each placed in a separate 100 mL volumetric flask. Then, the extract was added to the mixture. ethanol (pro analysis) as much as 100 mL (concentration 1000 μg/mL). Then, a series of concentrations of 20; 40; 60; 80; and 100 μg/mL was prepared. A total of 2 mL of each concentration series was pipetted and mixed with 2 mL of DPPH solution. 40 ppm. Mixture then homogenized And incubated in a dark room for 30 minutes. After incubation, absorbance was measured using spectrophotometry. UV-Vis with long wave 517nm with three repeats of [9].

- **Calculation % Inhibition And IC_{50}**

The percentage of antioxidant activity of ethanol extract of rosella flowers and essential oil of calamansi orange peel was calculated using the following formula:

$$\% \text{ inhibition} = \left(\frac{orbansi\ Blanko - Absorbansi\ sampel}{Absorbansi\ balnko} \right) \times 100\%$$

Calculation mark IC_{50} is done based on equality linear regression obtained from connection between concentration And percent inhibition Which declare a relationship between concentrations (x) and %inhibition (y) in Microsoft Excel. The formula is:

y = bx + c

y: % inhibition

x: Concentration sample

b: gradient (slope line) c: Intercept (y value when x=0)

Where to get mark IC50 moreover formerly searching for mark x with formula:

$$x = \frac{y-c}{b}$$

With substitute mark 50 to in y-axis on equality regression, the resulting x value represents the IC_{50} value [6].

3. Results

3.1. Yield Rosella Flower Petal Extract and Calamansi Orange Peel Essential Oil

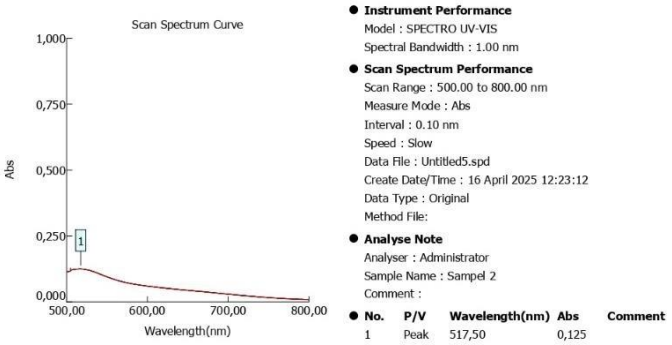
From 55.53 grams of powdered crude drug, an extract weighing 11.09 grams was obtained. The extract yield was 19.97%. Meanwhile, the yield of essential oil from calamansi orange peel was 1.61%. In this study, 24.135 grams of essential oil from calamansi orange peel were obtained from 1,500 grams of orange peel. The results can be seen in Table 1.

Table 1. Yield of rosella flower petal extract and calamansi essential oil

Sample	Initial Sample Weight (g)	Extract Weight (g)	Extract Yield
Rosella Flower Petals	55.53	11.09	19.97%
Kalamansi Orange Peel	15000	19.96	1.61%

3.2. Antioxidant Activity of Extract Combination

The maximum wavelength spectrum of DPPH solution was measured in the range 500-800 nm use spectrophotometer UV-Vis. The results of measuring the maximum wavelength of the DPPH solution using UV-Vis spectrophotometry obtained a maximum DPPH wavelength of 517.05 nm, which can be seen in Figure 1.



Picture 1. Spectrum Long Wave Maximum

Absorbance measurement results sample combination Rosella flower petal extract and calamansi orange essential oil with varying ratios of 1:1; 1:3; and 3:1 and vitamin C solution can be seen in Tables 2 until 5.

Table 2. Results Analysis Percentage Inhibition Combination Comparison 1:1

Replication	Absorbance Blank	Concentration	Absorbance	Percentage Inhibition	Regression linear	IC_{50}
1	0.126	20	0.074	41,26984	y = 0.5397x + 37,143 R= 0.9032	23,8224
		40	0.052	58.73015		
		60	0.024	80.95238		
		80	0.020	84.12698		
		100	0.022	82.53968		
2	0.124	20	0.079	36,29032	y = 0.5x +	20

		40	0.033	73.38709	40 R= 0.8196	
		60	0.026	79.03225		
		80	0.029	76.61290		
		100	0.019	84.67741		
3	0.123	20	0.078	36.58536	y = 0.561+ 36,748 R= 0.8887	23,6221
		40	0.038	69,10569		
		60	0.028	77.23577		
		80	0.020	83.73983		
		100	0.018	85.36585		

Table 3. Results Analysis Percentage Inhibition Combination Comparison 1:3

Replication	Absorbance Blank	Concentration	Absorbance	Percentage Inhibition	Regression linear	IC_{50}
1	0.126	20	0.106	15.87301	y = 0.8571x + 1.5873 R= 0.9868	56.4843
		40	0.080	36.50793		
		60	0.057	54.76190		
		80	0.030	76.19047		
		100	0.023	81.74603		
2	0.124	20	0.097	21.77419	y = 0.7984x + 6.4516 R = 0.9983	54.54458
		40	0.076	38.70967		
		60	0.057	54.03225		
		80	0.034	72.58064		
		100	0.019	84.67741		
3	0,123	20	0.096	21.95121	y = 0.7236x + 11.707 R = 0.9779	52.92012
		40	0.071	42.27642		
		60	0.051	58.53658		
		80	0.031	74.79674		
		100	0.027	78.04878		

Table 4. Results Analysis Percentage Inhibition Combination Comparison 3:1

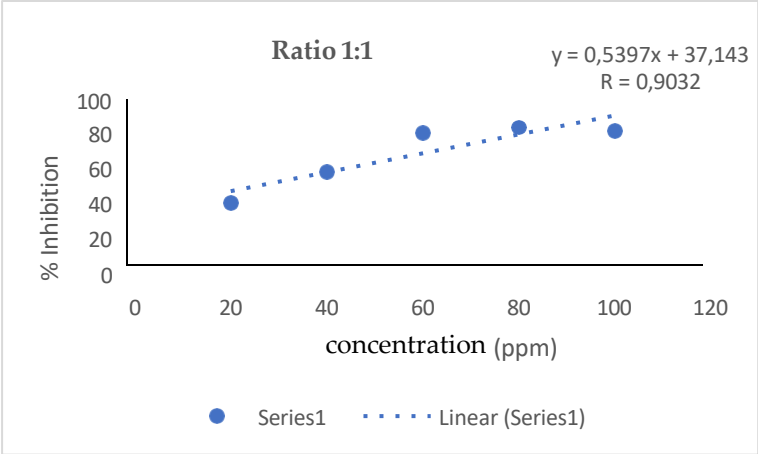
Replication	Absorbance Blank	Concentration	Absorbance	Percentage Inhibition	Regression linear	IC_{50}
1	0.126	20	0.068	46.0317	y=0.5x + 43.65 R= 0,9272	12.7
		40	0.039	69.0476		
		60	0.024	80.9523		
		80	0.021	83.34		
		100	0.014	88.89		
2	0.124	20	0.073	41.1290	y + 0.5565x + 38,871 R = 0.9280	19,998
		40	0.037	70.1612		
		60	0.030	75.8064		
		80	0.019	84.6774		
		100	0.013	89.51612		

3	0,123	20	0.078	36.5853	y = 0.5976x + 35,691 R= 0,8979	23,9441
		40	0.040	67.4796		
		60	0,022	82,1138		
		80	0,021	82,9268		
		100	0,014	88,61788		

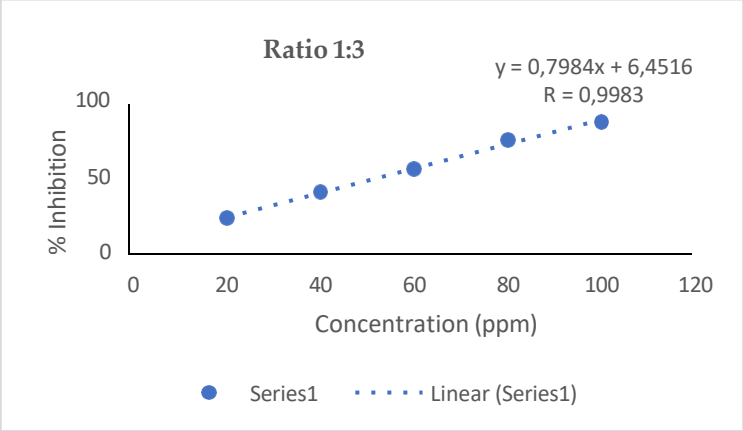
Table 5. Results Analysis Percentage Inhibition Control Positive Vitamin C

Replication	Absorbance Blank	Concentration	Absorbance	Percentage Inhibition	Regression Linear	IC_{50}
1	0.126	0.061	51,5873	0.061	y = 2.1429x+47.302 R= 0.9845	1.2590
		0.054	57.14285	0.054		
		0.052	58.73015	0.052		
		0.046	63.49206	0.046		
		0.038	69.84126	0.038		
2	0.124	0.068	45,16129	0.068	y = 2.8629x + *39,597* *R= 0.9911*	3.6337
		0.062	50	0.062		
		0.052	58,06451	0.052		
		0.045	63.70967	0.045		
		0.041	66.93548	*0.041*		
3	0,123	0.068	44.71544	*0.068*	y = 2.9945x + 38,022 R= 0.9893	4
		0.064	47.96747	0.064		
		0.052	57.72357	0.052		
		0.047	61.78861	0.047		
		0.040	67.74967	0.040		

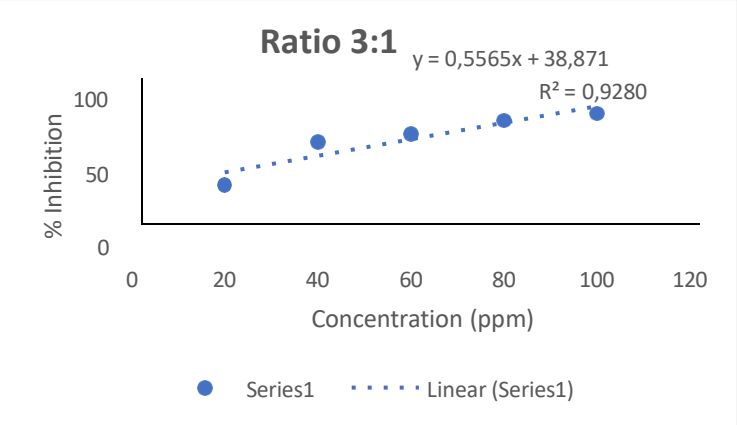
From the table that has been presented it can be seen that the more big mark concentration test so the more small absorbance obtained. This is inversely proportional to the increasing percentage of inhibition so that a linear regression can be obtained for each of them which can be seen in Figures 2 until 5 with the IC 50 value of the extract combination which can be seen in Table 6.



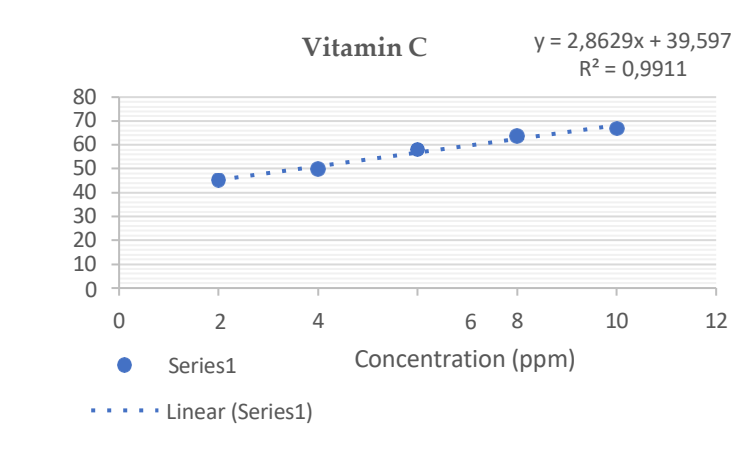
Picture 2. Regression Linear Curve of Combination 1:1



Picture 3. Regression Linear Curve of Combination 1:3



Picture 4. Regression Linear Curve of Combination 3:1



Picture 5. Curve Chart Regression Linear Vitamin C

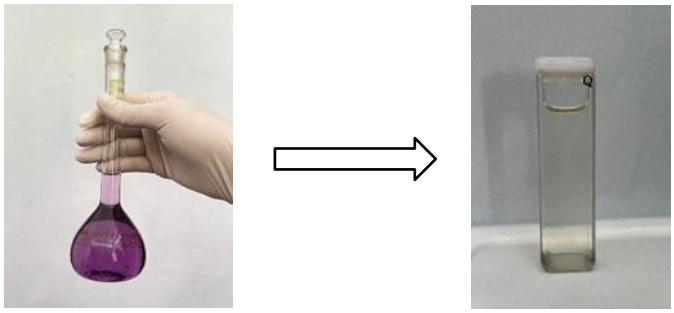
Table 6. Activities Antioxidants Combination Ethanol Extract Flower Rosella and Kalamansi Orange Essential Oil

Comparison	IC_{50} value			Mean±SD (ppm)	Activity Categories
	1	2	3		
1:1	23,8224	20	23,6221	22.48±2.151	Very strong
1:3	56,4843	54,54458	52.92012	54.64±1.784	Strong
3:1	12.7	19,998	23,9441	18.88±5.704	Very strong

4. Discussion

This study aims to determine the antioxidant activity value of a combination of rosella flower petal extract and calamansi orange essential oil using the DPPH *(1,1-diphenyl-2-picrylhydrazyl) method*. In this study, Absorbance measurements were carried out using a UV-Vis spectrophotometer at a wavelength of 517.50 nm.

Test activity antioxidants to combination extract petals flower The combination of roselle flower extract and calamansi orange essential oil was carried out by adding a DPPH solution at a concentration of 40 ppm. Antioxidant activity was indicated by a color change from purple to light yellow. The test yielded positive results for the combination of roselle flower extract and calamansi orange essential oil. The qualitative test results are shown in Figure 6.



Picture 6. Results Test Color Activity Antioxidants Sample

Qualitative analysis of the DPPH method is characterized by a change in the color of DPPH. The purple color is caused by the presence of release radical free resulting from reaction atom molecule DPPH. The presence of antioxidant activity is indicated by a change in the color of the solution from purple to yellow. old become color yellow. Solution sample Which homogenized with DPPH was incubated for 30 minutes in the dark, then the absorbance was measured using spectrophotometer UV-Vis on long wave maximum [6].

Antioxidants can be said to be strong, very strong or weak, which can be seen by measuring the IC50 value . sample. IC 50 measurement This test is carried out by determining the wavelength and determining the absorbance value of each sample. activity antioxidants in this study were carried out by combining 96% ethanol extract of flower petals rosella And oil Kalamansi orange peel essential oil with extract combination ratios of 1:1; 1:3; and 3:1. Extract concentration ratios such as 3:1, 1:1, and 1:3 were used to evaluate potential dominance each extract as well as detect interaction

between compound active, approach This is commonly used in combination antioxidant studies [10].

The ratio of extract combinations in absorbance measurement was made in 5 concentration series, namely 20, 40, 60, 80, and 100 ppm to determine the linear regression equation. Absorbance measurements were repeated 3 times so that the final calculation result is the average result of 3 different data sets. The results obtained by the correlation coefficient (R) showed different results. In the study, the R value obtained was >0.9. It means data has fulfil requirements. Concentration affects the percentage of inhibition, which is shown through equality y regression = bx + c . Value R is approaching or equal to 1 indicates a very strong correlation between concentration and inhibition percentage. The greater the correlation coefficient value, the closer the relationship between the two variables, and conversely, the smaller the correlation coefficient value indicates a weaker relationship [11]. Data linearity is acceptable if the R value is > 0.9 [12].

The antioxidant activity of a combination of 96% ethanol extract of rosella flower petals and essential oil of calamansi orange peel showed varying results depending on the ratio. 1:1 comparison obtained value IC 50 of 22.4815 μg/mL and a ratio of 3:1 of 18.8807 μg/mL, both of which have very strong antioxidant potential (IC 50 < 50 μg/mL). Meanwhile, at a ratio of 1:3 the value IC 50 as big as 54.6496 μg/mL classified as strong (50 μg/mL- 100 μg/mL). As a comparison, according to research conducted by Amriani and Tuahatu, 2021, the IC50 value Rosella flower petal extract and calamansi orange peel essential oil individually were 43 μg/mL and 50.31 μg/mL. Based on data the, combination flower extract rosella And oil essential oils show contribution positive And results Which better at a ratio of 1:1 and 3:1.

The ratio 1:3 of extract ethanol flower rosella and oil calamansi essential oil, the combination in this ratio shows lower antioxidant activity compared to extract his only one. This is thought to be caused by the dominance of essential oils, which are highly oxidizable. According to Abdelmohsen and Elmaidomy (2025), essential oils are highly susceptible to oxidation. experience oxidation, especially Because content compound not saturation which readily react with oxygen. This oxidation process forms unstable hydroperoxides, which then decompose into secondary products such as alcohols, aldehydes, ketones, and acids. These oxidation products not only alter the chemical characteristics of essential oils but can also significantly reduce or even eliminate their antioxidant properties, negatively impacting the resulting combination [13].

However, the results obtained were not superior to the antioxidant potential of Vitamin C, which was used as a positive control in this study. The activity antioxidants vitamin C classified as very strong with mark IC 50 2.96±1.488 μg/mL. Advantages this caused by properties of vitamin C as pure compound which has been known effective in neutralize radical free [14].

5. Conclusions

Based on the results of the research that has been conducted, it can be concluded that the IC_{50} value of the combination of 96% ethanol extract of rosella flower petals and essential oil of calamansi orange peel with a ratio of 3:1; 1:1; and 1:3 is 18.8807 respectively. μg/mL; 22,4815 μg/mL; and 54.6496 μg/mL . Based on the range of antioxidant activity from combination of roselle petal extract and calamansi essential oil antioxidant activity test, in 3:1 (18.8807 μg/mL) and 1:1 (22.4815 μg/mL) ratio have very

strong antioxidant activity, while in 1:3 (54.6496 μg/mL) ratio has strong antioxidant activity.

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