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Research Article

Antioxidant Activity of *Citrus nobilis var Microcarpa* and *Citrus aurantifolia* Hard Candy

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Abstract

In Indonesia, Pontianak oranges (*Citrus nobilis var Microcarpa*) and limes (*Citrus aurantifolia*) are commonly consumed as food and used in traditional medicine, with their antioxidant compounds that contribute to the protection of body cells from oxidative stress caused by free radicals. This research aimed to assess hard candy formulations using hedonic evaluation and to analyze the antioxidant activity of a blend of Pontianak oranges and limes. The evaluation comprised weight uniformity, stability, sugar reduction, and antioxidant activity analysis using the ABTS method. Antioxidant testing of Pontianak orange and lime juice was conducted using three formulations with ratios of 2:3 (F1), 3:2 (F2), and 1:1 (F3). The antioxidant activity values of the fruit juice formulations F1, F2, and F3 were 75,254 ppm, 87,141 ppm, and 79,223 ppm, respectively. In contrast, the corresponding hard candy formulations exhibited antioxidant activity values of 171,863 ppm, 209,729 ppm, and 180,805 ppm. The hard candy met the weight uniformity criteria, with no samples exceeding a 5% deviation from the average weight. The formulations were stable at temperatures of 8–15°C, with reducing sugar contents of 11,170%, 11,726%, and 11,377%.

Keywords: Pontianak orange, lime, antioxidant, evaluation

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1 Introduction

Antioxidants are compounds that can counteract the negative effects of free radicals in the body, where free radicals bound with antioxidant compounds will prevent the occurrence of various diseases [1]. Antioxidants can prevent the onset of degenerative diseases such as premature aging, cardiovascular diseases, hypertension, diabetes, and cancer. Several studies mention that antioxidants can be found in fruits and vegetables. One of the fruits high in antioxidants is citrus fruits such as Pontianak oranges and limes. Pontianak oranges are a type of Siamese orange that is quite widely distributed in Indonesia. This plant can spread in lowland areas up to an altitude of 770 meters above sea level. Pontianak oranges are also known as Siam oranges (*Citrus nobilis var Microcarpa*) which originate from the city of Pontianak, West Kalimantan. The pontianak orange contains 50,12 mg of vitamin C per 100 g and also contains antioxidants, carotenoids, and glucose [2]. Pontianak oranges have antioxidant activity in the category with an Inhibition Concentration 50% (IC_{50}) value of 18,542 mg/L [3].

Lime (*Citrus aurantifolia*) is one of the citrus fruits that has many benefits for both food and medicine. Lime is generally used as a flavor enhancer in cooking and to eliminate the fishy smell in fish processing. Traditionally, lime juice can be made into a drink to treat diseases such as inflammation and cough as well as respiratory tract disorders [4]. Lime contains vitamin C and has antibacterial activity [5]. Lime has strong antioxidant activity with an IC_{50} value of 49,589 mg/L [6]. Based on the description, the author is interested in making a hard candy preparation from the juice of Pontianak oranges and lime,and determining the antioxidant activity of the preparation. Hard candy preparations with a combination of Pontianak oranges and lime can be an alternative for consuming natural ingredients with a more delicious taste, practical for consumption, popular among various groups, and have high antioxidant activity.

2 Method

The research method used is a laboratory experiment, which involves observation, testing, or measurement activities in the Tropical Pharmacology Research and Development Laboratory, Faculty of Pharmacy, Mulawarman University, Samarinda, East Kalimantan, Indonesia. The materials to be studied are the juice of Pontianak oranges and lime, with additional ingredients used in the formulation of hard candy, namely sucrose, glucose syrup, and water. Sample collection was conducted in the Sanga-Sanga area, East Kalimantan. The laboratory experiment in this study was conducted by measuring the antioxidant activity of fruit juice and hard candy preparations using the ABTS method, and evaluating the hard candy preparations through weight uniformity tests, stability tests, and reducing sugar tests.

(1) Antioxidant activity testing, The procedure begins with the preparation of a 1000 ppm stock solution. Weighing each concentration variation of 10 mg, dissolved in methanol to a total volume of 10 mL in a volumetric flask. A series of solutions with concentrations of 5, 10, 20, 40, and 80 ppm were prepared. Then followed by the antioxidant activity test. The ABTS stock solution was diluted in a ratio of 1:4 with methanol for the blank measurement. 1 mL of the diluted ABTS stock solution and 1 mL of the concentration series solution were taken in a vial in the dark, then incubated for 30 minutes. Then, read the absorbance of antioxidant activity at the maximum wavelength. 1 mL of the diluted ABTS solution and 1 mL of the concentration series solution were taken to obtain the absorbance value [7].

(2) The weight uniformity test is conducted by weighing one sample of 10 preparations and then calculating the average weight of the preparations. The percentage deviation is calculated with the condition that no hard candy should have a weight deviation greater than 5% and none should have a weight deviation greater than 10%, stability test, and reducing sugar test [8]. (3) The stability test is conducted by storing each formula of hard candy in a container and keeping it for 2 weeks at cool temperatures (8-15°C) and room temperatures (23-30°C) with both open and closed packaging, then observing changes in color, texture, aroma, and taste of the preparation [9].

(4) The reducing sugar test is conducted by weighing 2 g of hard candy dissolved in aquades, transferring it into a 100 mL volumetric flask, and adding until the mark. 12,5 mL of the hard candy solution is taken into a 250 mL Erlenmeyer flask, then 40 mL of 3% HCl solution is added, and it is boiled for 1 hour with a vertical condenser. Cooled and neutralized with a 30% NaOH solution, then transferred

the contents into a 100 mL volumetric flask and adjusted the volume with aquades up to the mark, then filtered. After that, 10 mL of the filtrate is pipetted into a 250 mL Erlenmeyer flask, then 25 mL of Luff Schoorl solution and a few boiling stones, as well as 15 mL of aquades, are added. Then heat the mixture and ensure that the solution boils within 3 minutes. Boil for exactly 10 minutes and then quickly cool with running water. Add 15 mL of 30% KI solution and 25 mL of 25% H_2SO_4 solution carefully, then titrate with 0,1N $Na_2S_2O_3$ solution until a light yellow color appears. Add 2 mL of 1% starch indicator, then titrate again with sodium thiosulfate until a color change occurs in the turbid white solution [10].

3 Result and Discussion

Antioxidant testing was conducted using the ABTS free radical soaking method or 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid. The principle of the ABTS method test is to measure the antioxidant activity against the ABTS free radical, which is indicated by the decrease in the intensity of the color of the ABTS radical. The reason for using ABTS is that the ABTS method is easy and quick to perform, requiring only a small amount of sample and having a relatively high sensitivity [11]. The antioxidant activity test was conducted using p.a methanol solvent because methanol does not affect the reaction between the test sample as an antioxidant and ABTS as a free radical [12]. The parameter for the antioxidant activity test results using the ABTS method is the IC_{50} value. It is considered a very strong antioxidant if the IC_{50} value is less than 50 ppm, strong for an IC_{50} value of 50-100 ppm, moderate if the IC_{50} value is 101-150 ppm, weak if the IC_{50} is 151-200 ppm, and very weak if the IC_{50} >200 ppm. This indicates that the lower the IC_{50} value of a sample, the greater its antioxidant activity [13].

Based on the research results, the three concentrations of Pontianak orange and lime juice have different antioxidant activities, as presented in Table 1. At the concentration of Pontianak orange and lime juice (2:3), an IC_{50} value of 75,254 ppm was obtained, at the concentration (3:2), an IC_{50} value of 87,141 ppm was obtained, and at the concentration (1:1), an IC_{50} value of 79,223 ppm was obtained. The results of the antioxidant test show that the juice of Pontianak oranges and lime at all three concentrations have antioxidant activity that falls into the strong category, because the IC_{50} values obtained are in the range of 50-100 ppm. and in the antioxidant activity of the hard candy presented in the table, the (2:3) and (1:1) ratios have weak antioxidant activity, while the (3:2) ratio has very weak antioxidant activity.

Table 1. Antioxidants in the juice of Pontianak orange and lime

Formulation	Concentration	Absorbance	% inhibition	Equation	IC_{50}
2:3	5	0,488	30,975	Y=0,2577x + 30,607 R^2=0,9585	75,254
	10	0,475	32,767		
	20	0,456	35,502		
	40	0,397	43,752		
	80	0,353	49,976		
3:2	5	0,496	29,797	Y=0,2347x + 29,548 R^2=0,9753	87,141
	10	0,479	32,201		
	20	0,468	33,804		
	40	0,418	40,782		
	80	0,371	47,524		
1:1	5	0,484	31,447	Y =0,2498x + 30,21 R^2=0,9927	79,223
	10	0,473	33,050		
	20	0,464	34,276		
	40	0,417	41,018		
	80	0,353	49,976		

At the concentration of hard candy made from Pontianak orange and lime juice in table 2, the (2:3) formulation obtained an IC_{50} value of 171,863 ppm, the hard candy with a (3:2) concentration obtained an IC_{50} value of 209,729 ppm, and the (1:1) concentration obtained an IC_{50} value of 180,805 ppm. Based on the three concentrations, the (2:3) and (1:1) ratios have weak antioxidant activity, while the (3:2) ratio has very weak antioxidant activity [13]. The difference in antioxidant activity of each formulation can be influenced by the varying concentrations of Pontianak orange and lime juice. Furthermore, the higher the concentration of lime, the more antioxidant compounds there will be that act as hydrogen or electron donors to free radical compounds [14]. The ripeness level of Pontianak oranges and limes can affect the vitamin C content, where vitamin C is one of the antioxidant compounds, and if its amount decreases, the antioxidant activity also decreases. The vitamin C content decreases because the vitamin C in overripe fruit will convert into glucose. In this study, a higher concentration of Pontianak oranges resulted in decreased antioxidant activity because the Pontianak oranges used were overripe [15].

This testing is followed by the evaluation of the preparation, namely weight uniformity, stability, and reducing sugar. The weight uniformity test aims to determine the uniformity of the preparation and to ensure that each hard candy contains a certain amount of extract and additives according to the specified dosage. The weight uniformity test is conducted to observe the uniformity of the amount of orange fruit extract and other ingredients that enter the body, so that the content of each preparation is expected to be the same and in accordance with food safety standards. Indirectly, weight uniformity indicates the uniformity of content within a preparation [16].

Table 2. Antioxidant Hard Candy

Formulation	Concentration	Absorbance	% inhibition	Equation	IC_{50}
2:3	5	0,479	30,281	Y=0,1122x + 30,717 R^2=0,9604	171,863
	10	0,467	32,025		
	20	0,456	33,624		
	40	0,442	35,755		
	80	0,417	39,292		
3:2	5	0,486	31,494	Y=0,0866x + 31,838 R^2=09489	209,729
	10	0,475	32,814		
	20	0,467	33,899		
	40	0,452	36,067		
	80	0,436	38,330		
1:1	5	0,489	28,875	Y=0,2498x + 29,334 R^2=0,9455	180,805
	10	0,479	30,329		
	20	0,462	32,751		
	40	0,451	34,399		
	80	0,426	38,032		

Based on the results of the average weight uniformity test, the hard candy F1 is 2,858 g; F2 is 2,859 g, and F3 is 2,870 g. All three formulations of hard candy meet the weight uniformity test requirements because in each formulation, there should not be a single preparation that deviates from the percentage deviation (Pharmacope edition VI, 2020). The use of molds affects the weight of the preparation where the mold shape and size are the same. Uneven weight can be influenced during the pouring process into the mold if it is not done evenly. The addition of glucose syrup makes the hard candy solution thick, making it quite difficult to pour and resulting in uneven candy weight [17]. The results of the hard candy preparation evaluation tests, namely the weight uniformity test, can be seen in Table 3.

Table 3. Weight Uniformity Test

Value	Weight		
	F1	F2	F3
Average	2,858	2,859	2,870
A±5%	2,715-3,001	2,716-3,002	2,727-3,013
A±10%	2,572-3,144	2,573-3,145	2,583-3,157
Conclusion	Q	Q	Q

Note : Q (qualified)

Stability testing is conducted to determine the stability of hard candy during its storage process. Stability tests were conducted over 2 weeks with different temperatures for each formulation, and organoleptic changes in the hard candy were observed with parameters of color, aroma, texture, and taste. Observation results over 2 weeks showed that F1, F2, and F3 stored at temperatures of 8-15°C with both closed and open packaging remained stable. For F1, F2, and F3 preparations stored at temperatures of 23-30°C in closed packaging, the candy preparations were stable, whereas in open packaging, the preparations were unstable. Unstable hard candy preparations are characterized by the hard candy undergoing a change in shape from initially solid to slightly melted [18]. The growth of mold is caused by the influence of glucose content and moisture in the formula. Glucose is used by microbes as nutrition and a growth medium, so the higher the glucose content, the more microbes will grow. The stability test can be seen in Table 4. Other ingredients such as glucose syrup, which serves as a sweetener, can increase the risk of contamination and microbial growth in the preparation. Another factor that influences is the environmental factor, namely storage temperature and packaging. Poor storage and packaging will affect the interaction of hard candy with external air, leading to cross-contamination that will reduce the physical stability of the preparation [19]. Unstable hard candy preparations can also be caused by the fact that in this study, the preparations did not use food preservatives, thereby triggering microbial growth [20].

Table 4. Stability Test for 2 Weeks

Organoleptic	Temperature	Packaging	F1	F2	F3
Taste	8-15°C	Closed	S	S	S
		Open	S	S	S
	23-30 °C	Closed	S	S	S
		Open	S	S	S
Aroma	8-15°C	Closed	S	S	S
		Open	S	S	S
	23-30 °C	Closed	S	S	S
		Open	S	S	S
Color	8-15°C	Closed	S	S	S
		Open	S	S	S
	23-30 °C	Closed	S	S	S
		Open	S	S	S
shape	8-15°C	Closed	S	S	S
		Open	S	S	S
	23-30 °C	Closed	U	U	U
		Open	S	S	S

Notes : S (stable); U (unstable)

The reducing sugar content of hard candy F1 is 11,170%, F2 is 11,708%, and F3 is 11,377%. The allowable reducing sugar value based on SNI 3547.1:2008 is a maximum of 24%, so hard candy F1, F2, and F3 comply with the standard. The level of sugar reduction can be influenced by the ratio of sucrose to glucose syrup and the cooking process. If the reducing sugar content exceeds 24%, it can cause stickiness or graining [21]. Based on the results obtained, F2 has a higher reducing sugar content compared to F1 and F3, because F2 contains more juice from Pontianak oranges compared to F1 and F3. It is known that Pontianak oranges have a sweeter taste than lime, so the glucose content in Pontianak oranges is higher, resulting in a higher reducing sugar value for F2. The addition of Pontianak oranges to sweet hard candy also accelerates the inversion of sucrose into glucose and fructose. The high sweetness and sucrose content are what cause the high reducing sugar levels in the preparation [22]. The reducing sugar test can be seen in Table 5.

Table 5. Reducing Sugar Test

Evaluation	Formula			SNI Quality Standard 3547.1:2008
	F1	F2	F3	
Reducing Sugar (%)	11,170±0,220	11,726±0,119	11,377±0,106	Maks. 24%

4. Conclusion

Based on the research conducted, the antioxidant activity of the juice from Pontianak oranges and lime at concentrations (2:3), (3:2), and (1:1) has an IC_{50} value of less than 100 ppm, which falls into the strong category. the antioxidant activity of hard candy from Pontianak orange and lime juice at concentrations (2:3), (1:1) has an IC_{50} value of less than 200 ppm, which falls into the weak category, and (3:2) has an IC_{50} value of more than 200 ppm, which falls into the very weak category. Based on the evaluation results, the weight uniformity test showed that none of the formulations had a weight deviation, and the reducing sugar test (< 24%) for each hard candy formula complied with the Indonesian National Standard (SNI). The formulations were also quite stable at temperatures of 8-15°C compared to 23-30°C. Based on hedonic testing, formulation F1 was the most preferred by the panelists.

5. Declarations

5.1 Acknowledgements (Optional)

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5.2 Author contributions

Each author listed in this journal contributed to the conduct and completion of this research.

5.3 Conflict of Interest

The authors confirm that there are no conflicts of interest associated with the conduct or publication of this research.

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