



The Effect of Simplisia Drying Method on Antioxidant Activity of Senggani Fruit Extract (*Melastoma Malabathricum L.*) by DPPH (2,2-Diphenyl-1-picrylhydrazyl)

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Abstract

Senggani fruit (*Melastoma Malabathricum L.*) contains anthocyanin that functioning as an antioxidant. Anthocyanin are tremendously sensitive to thermal processes which trigger phytochemical or photo-oxidation reactions that can open anthocyanin rings. The objective of this study is to identify the effect of the simplicia drying method on the antioxidant activity of Senggani fruit extract. Senggani fruit extract was prepared by obtaining samples of ripe fruit, dry sorting, washing, wet sorting, and drying using two methods; sunlight and oven at 70°C. After the simplicia was dry, it was blended and sifted until smooth. The fine simplicia was macerated with 96% ethanol and evaporated to gain a crude extract. The crude extract was assessed with reagents for phytochemical screening. Furthermore, the crude extract was examined for antioxidant activity by the DPPH method. This study implies that the simplicia and crude extract of Senggani fruit from drying in sunlight and oven possess different organoleptic properties such as color, smell, and taste. In phytochemical testing with reagents, it was discovered that anthocyanin compounds were unveiled in drying utilizing sunlight while employing an oven at 70°C; no anthocyanins were found. The antioxidant testing of Senggani fruit extract revealed that the drying method employing sunlight had an IC₅₀ value of 18.8 g/mL while the oven temperature of 70°C owned an IC₅₀ value of 28.3 g/mL. Based on the study results, it can be identified that the simplicia drying method affects the antioxidant activity of the Senggani fruit extract. The drying method in the sun produces extracts with greater antioxidant activity while drying in an oven at 70°C results in a degradation process of anthocyanin compounds, thereby decreasing the antioxidant activity of the Senggani fruit extract.

Keywords: *Melastoma Malabathricum L.*, Drying Method, Antioxidant Activity.

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1. INTRODUCTION

People have long employed various types of plants as medicine. Indonesia possesses more than 1,000 species of plants which can be applied as medicine and about 300 species that have been utilized for traditional medicine (Handayani & Rusmita, 2017). The implementation of medicinal plant species is indeed employed for generations, and it is an effort to preserve plant cultivation in the traditional medicine field (Pitoyo & Triwahyudi, 2017).

Senggani is one of the most beneficial weeds. The fruit, flowers, and leaves of this plant are extensively used for medicine and natural food coloring (Ondagau, et al., 2018). Senggani fruit possesses active compounds that can be utilized as traditional medicine. The profound compound in the Senggani fruit is anthocyanin which is a flavonoid derivative compound. Senggani fruit is well-known to possess active ingredients as a source of antioxidants (Kartikasari & Ropiqa, 2018).

The utilization of plants as traditional medicine employs simplicia and crude extract from plants. One of the post-harvest processes that play a significant role in the quality of simplicia is the drying process (Departemen Kesehatan Republik Indonesia, 2000). The drying process and method affect the chemical compounds content and pharmacological effects in a medicinal plant, particularly compounds which are efficacious as antioxidants. Previous research was conducted to determine the drying effect of Senggani leaf administering ovens and dry wind. Research displays the antioxidant activity of oven drying Senggani leaf extract 52.76% and dry wind 54.60% (Luliana, et al., 2016). In this study, the part of the plant employed to measure the difference in antioxidant activity was the fruit in addition to the parameters examined to be IC50 values.

Anthocyanins contained in Senggani fruit are tremendously sensitive to thermal processes. The color disappears and turns to brown as the pigment is degraded and polymerized (Amperawati, et al., 2019). Light and heat degrade anthocyanin pigments and generate colorless chalcones. The energy released by light triggers phytochemical or photo-oxidation reactions to open the anthocyanin ring. Longer exposure causes further degradation and other derivative compounds formation such as 2,4,6 trihydroxy benzaldehyde and substituted benzoic acid (Alappat & Alappat, 2020).

2. RESEARCH METHOD

The tools and materials utilized in this study comprise of desiccator, thermometer, rotary evaporator, hotplate, water bath, UV-Vis 1800 Shimadzu spectrophotometer, 96% ethanol, iron (III) chloride, magnesium powder, concentrated hydrochloric acid, sodium hydroxide, Lieberman-Burchard reagent, DPPH powder.

Senggani plants were obtained from Rawung Village, Tangkiling, Bukit Batu District, Central Kalimantan. The part of the Senggani plant sampled in this study was the ripe fruit. Senggani plants were determined in full on plant parts at the Plant Systematics Laboratory, Faculty of Biology UGM, numbered 014940/S. Tb/I/2021.

Senggani fruit powder was macerated with 1:3 ethanol 96% solvent every 24 hours, filtered to obtain the filtrate, replaced with new ethanol, and repeated for three days, then evaporated utilizing a rotary evaporator to produce a crude extract (Sastrawan et al., 2013).

Phenolic test: Administering 1N FeCl_3 solution as a reagent. The addition of FeCl_3 extract produces a blackish green color. This color is generated because phenol compounds react with Fe^{3+} ions to compose complex compounds (Luliana, et al., 2016).

Flavonoid test: crude extract was obtained 2 mL, added 0.1 g of magnesium powder, then added ten drops of concentrated HCL, and shaken slowly. A positive test revealed a red-orange to red-purple color (Sastrawan et al., 2013).

Anthocyanin test: Positive extracts containing flavonoid group compounds were administered with 2N HCl heated at 100°C for 5 minutes. Positive results are obtained when a red color appears. NaOH 2 N was also added drop by drop while observing the color changes occurring. Positive results are obtained when a blue-green color occurs, which fades slowly (Ondagau, et al., 2018).

Saponin test: Crude extract of the Senggani fruit was placed into a test tube. Then, 10 mL of hot and cold water was added and then shaken vigorously for 10 seconds. If it is positive, a solid foam appears for 10 minutes, as high as 1 cm – 10 cm. If 2N HCl is added, the foam appears as well (Martiningsih, et al., 2016).

Triterpenoid & steroid test: Using Lieberman–Buchard reagent (concentrated acetic anhydrous sulfuric acid). A green color change implies positive results in this test for steroids and reddish-brown color for triterpenoids. The color change occurs due to the terpenoid/steroid compound group oxidation through the conjugated double bonds formation (Martiningsih, et al., 2016).

Determining the antioxidant activity of Senggani fruit extract as assessed by the method of Gaulejac et al. (1998), which regulates. A mixed solution (2:1) of 1,1-diphenyl-2-picrylhydrazyl (DPPH) in 0.1 mM methanol with an extract solution of 0.01 mg/mL concentration; 0.02 mg/mL; 0.03 mg/mL; 0.04 mg/mL; and 0.05 mg/mL were prepared. The degree of color reduction of the solution illustrates the radical scavenger efficiency. In the last five minutes of the 30 minutes, the absorbance was calculated by spectrophotometer at λ 517 nm (Damanis, et al., 2020; De Gaulejac, et al., 1999).

$$\text{Antioxidant activity (\%)} = \frac{1 - \frac{\text{sample absorbance}}{\text{control absorbance}}}{100\%}$$

3. RESULTS AND DISCUSSION

Simplicia of Senggani fruit was formulated by two drying methods, sunlight, and oven at 70°C. The objective of the two drying methods was to observe the effect of the drying method on the simplicia, extract, and content of the obtained secondary metabolites. The organoleptic test of simplicia powder was administered to determine the distinctive physical properties of the plant by observing the specificity of the shape, color, smell, and taste of simplicia (Kementerian Kesehatan Republik Indonesia, 2017).

The organoleptic test in Table 1 presents the differences between oven-dried simplicia powder and sunlight in the form of shape, color, taste, and aroma. The simplicia powder dried in the sun provided better results because the simplicia powder possessed a sweet taste and a distinctive aroma of Senggani fruit. In contrast, the oven-dried one owned a slightly sweet taste, and there was no distinctive aroma of Senggani fruit. It is because heating with high temperatures or for a long time may result in physical and biochemical changes, thereby reducing the quality of the resulting product.

Senggani fruit crude extract was obtained by cold extraction by implementing the maceration method (Che Omar, et al., 2013). The maceration method is employed because the equipment and machining techniques are relatively simple and easy to conduct. Furthermore, the maceration method is administered to keep the secondary

metabolites of Senggani fruit from being damaged by the heat. 96% ethanol solvent was administered because it is non-toxic and semi-polar, hence, more secondary metabolites were extracted by the solvent. Ethanol solvent encompasses good absorption and is able to inhibit mold growth and germs (Mardina, 2011). It is demonstrated in Table 1 that the comparison of crude extract between oven drying and sunlight was conducted by organoleptic testing. The crude extract obtained by the oven drying method possesses a darker color and is odorless, while the viscous extract obtained by sun-drying owns a lighter color and a distinctive aroma of Senggani fruit.

Table 1. Organoleptic Test of Simplicia and Crude Extract.

No	Drying Method	Organoleptic			
		Consistency	Colour	Taste	Odor
Simplicia					
1.	Oven	Slightly coarse powder	Dark brown	Bittersweet	No odor
2.	Sunlight	Fine powder	Light brown	Sweet	Typical Senggani fruit
Crude Extract					
1.	Oven	Thick	Dark brown	-	No odor
2.	Sunlight	Thick	Light brown	-	Typical Senggani fruit

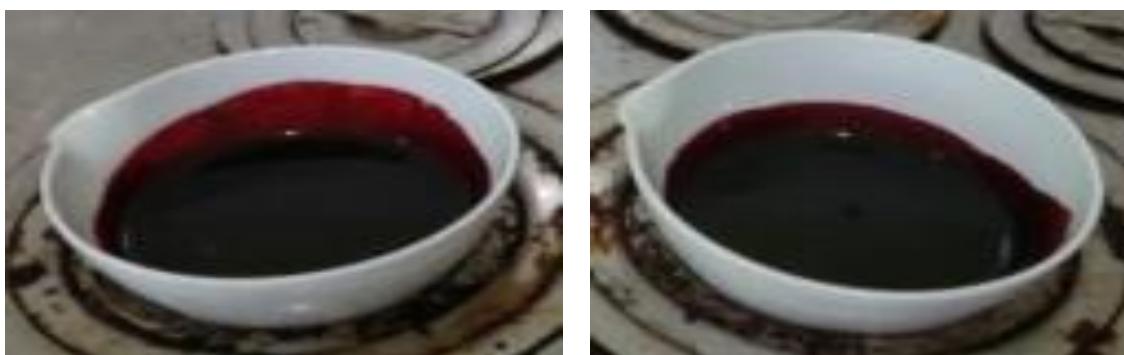


Figure 1. Color of Senggani fruit extract with drying treatment, A: sun-drying treatment; B: oven drying treatment

Phytochemical testing was utilized to determine quickly and simply whether a plant contains certain bioactive compounds or not (Danladi, et al., 2015). This test was performed employing chemical reactions. The results of this study presented that there were differences in the phytochemical test results of the ethanol extract of Senggani oven drying and sun drying. In oven drying, anthocyanin compounds were not discovered or negative. It is because during the simplicia drying process, it utilized an oven administering a high temperature so that the natural dyes from the Senggani fruit are lost due to not being able to withstand high heating (Kho, et al., 2017).

Moreover, steroid and triterpenoid compounds were not unveiled in the extract of the Senggani fruit. Different sampling locations significantly affected the results. The soil structure of a region is different so that it affects the results compared to previous studies explaining that the ethanol extract of Senggani fruit with different species

(*Melastoma affine* D. Don) contains steroids and triterpenoids. The ethanol extract of the Senggani fruit was sun-dried and oven-dried in that it did not contain steroid and triterpenoid compounds. Furthermore, in this study, it was revealed that the ethanol extract of Senggani fruit possessed saponins. In contrast, the ethanol extract of Senggani fruit with another species (*Melastoma affine* D. Don) did not possess saponins (Syafitri, 2014).

Table 2. Phytochemical Test Results of Secondary Metabolite Compounds.

Phytochemical Test	Treatment	Colorreaction	Result (+/-)		Result (+/-)	
			Sunlight	Oven	I	II
Phenolic	Added FeCl_3	Dark-green			+	+
Flavonoid	Added concentrated HCl	Red-orange red-purple	+	+	+	+
Anthocyanin	Added Mg powder and 2N HCl, heated at 100 °C and added 2N NaOH	Blue-green color fading slowly	+	+	-	-
Saponin	Added hot water shake vigorously	A foam lasting ± 10 minutes	+	+	+	+
Steroid	Added Lieberman Buchard	Green color	-	-	-	-
Triterpenoid	Added Lieberman Buchard	Sorrel	-	-	-	-

Description: Positive (+) contains a group of compounds.

Negative (-) does not contain a group of compounds.

The antioxidant activity of Senggani fruit extract was examined employing the DPPH method. The DPPH method (2,2-diphenyl-1-picrylhydrazyl) was selected because it is simple, easy, fast, and sensitive and merely requires a small sample to evaluate the antioxidant activity of the compound (Kedare & Singh, 2011). The principle of the DPPH method is to assess the DPPH radical capture by a compound with an antioxidant activity administering UV-Vis spectrophotometry. Hence, the value of free radical scavenging activity is identified. The result is expressed by the IC_{50} (Inhibitory Concentration) value (Damanis, et al., 2020). The IC_{50} value is defined as the concentration of the test compound which is able to decrease free radicals by 50% (Al Ridho, 2013). The antioxidant testing result employing the DPPH method on both oven-drying and sun-drying Senggani fruit extracts revealed differences in antioxidant activity by perceiving at the IC_{50} value. The different IC_{50} values of Senggani fruit extract with sun and oven drying are demonstrated in table 3.

Table 3. Antioxidant Activity of Senggani Fruit Extract.

Sample drying Method	Concentration (mg/mL)	Absorbance			% Average inhibition	Regression Equation	IC_{50}
		I	II	III			
Sunlight	0.010	0.585	0.590	0.579	37.20	$\text{Y} = 1283\text{x} +$ 25.87	0.0188 mg/mL 18.8
	0.020	0.448	0.455	0.442	51.84		
	0.030	0.312	0.309	0.316	66.45		
	0.040	0.180	0.205	0.196	79.20	$\text{R}^2 = 0.991$	$\mu\text{g/mL}$
	0.050	0.110	0.115	0.112	87.93		
Oven	0.011	0.762	0.766	0.756	18.22	$\text{Y} = 1680\text{x} +$	0.0283

0.021	0.575	0.581	0.553	38.81
0.032	0.387	0.385	0.401	58.00
0.042	0.235	0.242	0.244	74.29
0.053	0.095	0.102	0.105	89.29

Table 3 presents the IC_{50} test values for Senggani fruit extract, which was treated with sun drying. The value of the regression equation obtained from the antioxidant activity test was $y = 1283x + 25.87$ with a value of $R^2 = 0.991$ for the extract which simplicia was provided a sun-dried, and the regression equation for the extract which simplicia was provided an oven-dried treatment was $y = 1680x + 2.491$ with a value of $R^2 = 0.995$. The y-coefficient in the equation is the IC_{50} value of the Senggani fruit ethanol extract , while the x-coefficient in the equation is the concentration amount of the Senggani fruit extract that identified, in which x is the concentration required to reduce 50% of DPPH activity. The value of R^2 indicates the level of linear correlation in a test (> 0.990). The R^2 value in the regression results revealed a positive value. It means that the higher the concentration of the Senggani fruit extract, the greater the antioxidant activity (International Conference on Harmonisation, 1994). Linear regression curve graphs of Senggani fruit extract concentration (oven and sun drying) versus antioxidant activity can be perceived in Figure 2 and Figure 3.

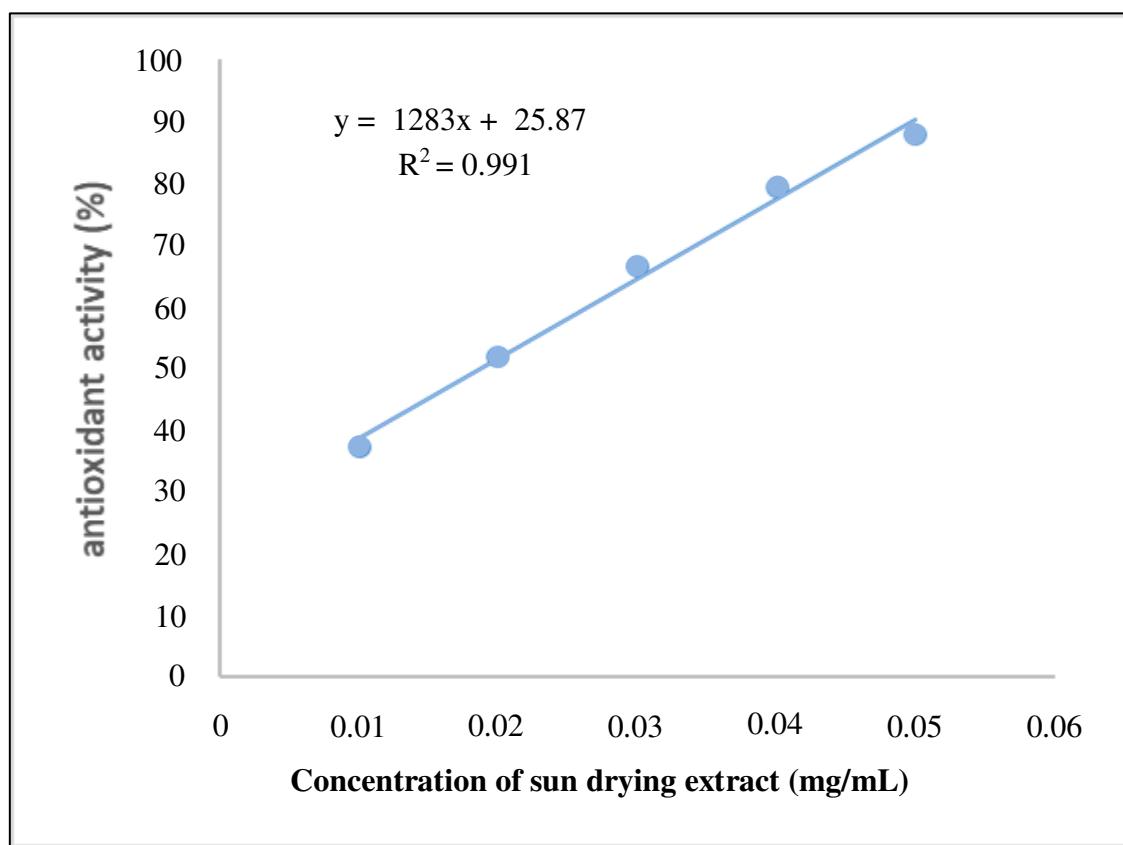


Figure 2. Regression curve of sun-dried Senggani fruit extract

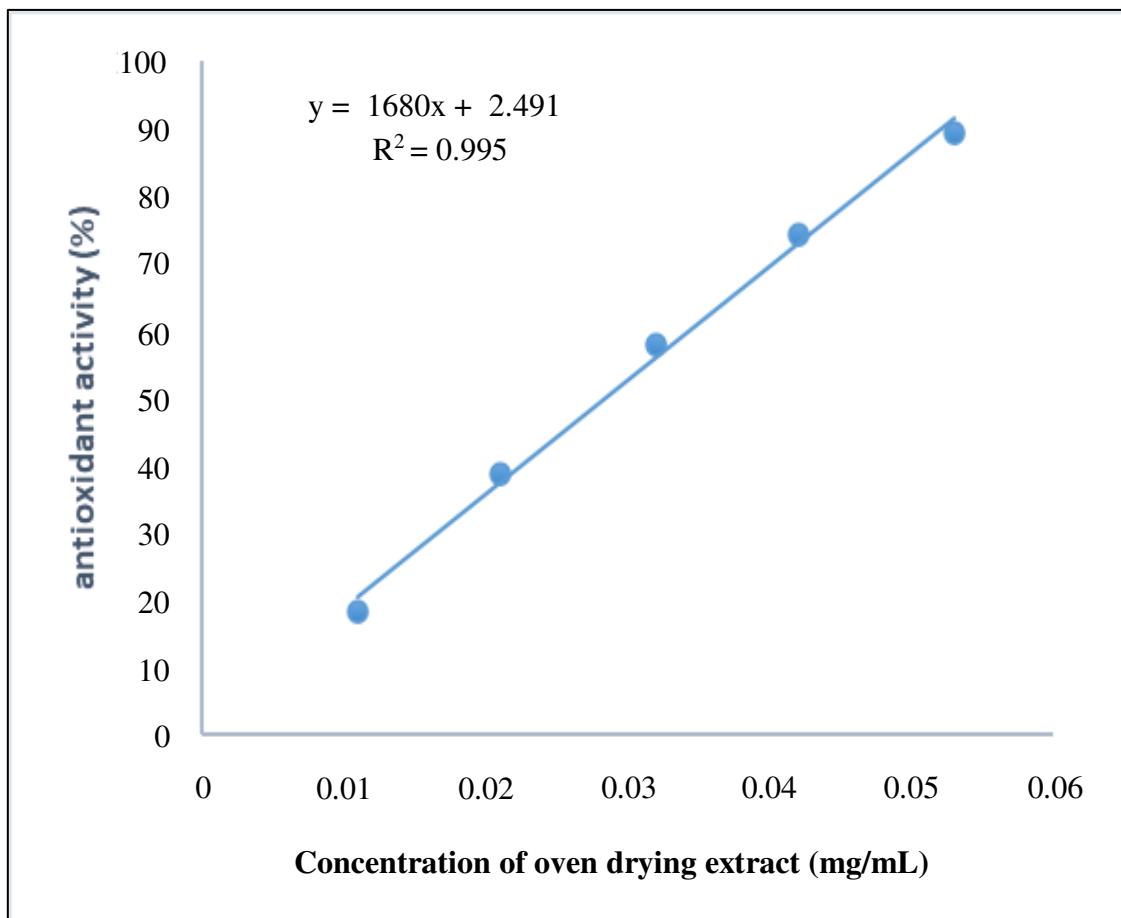


Figure 3. Regression curve of oven-drying Senggani fruit extract

The antioxidant activity of the Senggani fruit extract provided different treatment between sun drying and oven drying revealed different IC_{50} values. The greater the antioxidant activity of the sample calculated, the smaller the IC_{50} value. Sun-dried Senggani fruit extract possessed a greater antioxidant activity with an IC_{50} value of 18.8 $\mu\text{g/mL}$, implying that the power of sun-dried Senggani fruit extract could decrease 50% DPPH free radical activity with a concentration of 18.8 $\mu\text{g/mL}$. In contrast, the oven-dried Senggani fruit extract possessed a smaller antioxidant activity with an IC_{50} value of 28.3 $\mu\text{g/mL}$, indicating that the strength of oven-dried Senggani fruit extract could reduce 50% DPPH free radical activity with a concentration of 28.3 $\mu\text{g/mL}$.

Oven drying with a temperature of 70°C based on the research results is able to decrease antioxidant activity up to 66.43%. It is caused by the degradation of the active compound acting as an antioxidant in the extract of the Senggani fruit, anthocyanins because these compounds are susceptible to heating or thermolabile (Amperawati, et al., 2019). Anthocyanin degradation occurring due to temperature will change the chemical structure by opening the main anthocyanin ring into chalcone and transform the compound's color from purple to brown (Alappat & Alappat, 2020). The degradation of anthocyanins by thermal processes is demonstrated in Figure 4.

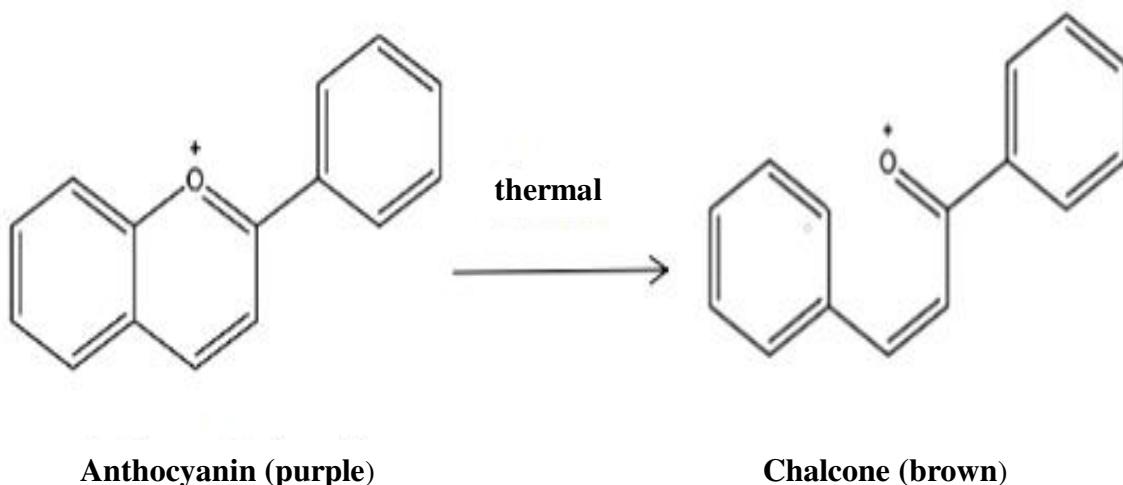


Figure 4. Degradation of anthocyanin compounds.

4. CONCLUSION

The antioxidant activity of Senggani fruit extract was affected by the simplicia drying method. Extract drying utilizing sun light possessed a greater antioxidant activity with an IC_{50} value of 18.8 $\mu\text{g/mL}$, while extract drying in an oven at 70°C possessed a smaller antioxidant activity with an IC_{50} value of 28.3 $\mu\text{g/mL}$.

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