SECONDARY METABOLITE AND ANTIOXIDANT ACTIVITY OF SOURSOP (ANNONA MONTANA) FRUIT EXTRACT

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ABSTRACT: Soursop (Annona montana) is one of the plants that can be used as an antioxidant because it contains terpenoid. Antioxidants are responsible for neutralizing the increase of free radicals. The purpose of this research is to determine the secondary metabolite and antioxidant activity using the DPPH free radicals. This research was included as a descriptive research, which was conducted in the Pharmacognosy Laboratory of Academy of Pharmacy Putra Indonesia Malang. The research stages include the collection of materials, determination of plant, extraction with the maceration method for 5 days using ethanol 70%, qualitative testing of secondary metabolite compounds, testing of antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH solution concentration is 100.9 ppm, while the sample concentrations are 60 ppm, 70 ppm, 80 ppm, 90 ppm, and 100 ppm dissolved with ethanol pa and replicated for 3 times. All the solutions were measured their absorbance using the UV-Vis spectrophotometer at 515 nm of wavelength. The finding shows that the secondary metabolite of terpenoid was found on the soursop fruit extract. Also, the IC_{50} value of 61 ppm was obtained. It can be concluded that the soursop fruit sample of 61 ppm can reduce the free radical level by 50% so it is categorized as a strong antioxidant.

Keywords: Antioxidant Activity, Annona Montana, DPPH, Secondary Metabolite.

1. Introduction

Nowadays, people often use plants as medicines for the treatment of certain diseases. Yet, there are some plants that have not been proven capable of curing some diseases, such as soursop (Annona montana). This plant has the same family with common soursop (Annona muricata), therefore it has the same fruit texture. Yet, there are differences in the forms of flesh color, fruit shape, seed color, and leaf size. People do not consume this plant fruit since the research on the safety consumption has not been conducted. Besides, the fruit does not have any taste or plain, but it has a fragrant aroma when ripe. In addition, the plant can grow fast along the year. According to Bratasasmita (2011), in every 100 grams of soursop (Annona muricata), there are 20 milligrams of antioxidant. As mostly understood, the vitamin C of soursop can be used to boost the body immune towards diseases. Some examples of important saponin components are polyphenol and flavonoid. Polyphenol and flavonoid are a natural antioxidant that works together.

This research was conducted from the preparation of soursop (Annona montana) until the extraction using ethanol as a dissolvent. The ethanol was selected because it is a universal dissolvent that can attract compounds dissolved in nonpolar and polar dissolvent. The ethanol also has the ability to dissolve a big amount of extract with a significantly different density so that it is easy to isolate the dissolved substances. Then, the phytochemical testing was conducted qualitatively on the soursop (Annona montana) fruit extract consisting of flavonoid, alkaloid, saponin, and terpenoid secondary metabolite testing. One of the methods that can be used is the phytochemical screening method (Harborne, 1987). It is the first step to identify the chemical contents in plants since it can determine the chemical compound group of the analyzed plants. The phytochemical screening on the simplisia powder and wet sample include a

compound content examination of alkaloid, flavonoid, terpenoid/steroid, tannin, and saponin according to the Harborne procedure (Harborne, 1987).

Based on the previous information, the antioxidant activity testing using 2,2-diphenyl-1-picrylhydrazyl (DPPH) was conducted. The DPPH method was selected because it is simple, easy, fast, and sensitive. Also, it only needs a small amount of sample. The testing aims to determine the antioxidant activity on soursop after the phytochemical testing. The method was conducted by the observation of the DPPH radical capture by the visible spectrophotometry. The anti-free radical testing using the DPPH method resulted in the data that were processed using the % inhibition formula. In Indonesia, the research of secondary metabolite and antioxidant testing on soursop (Annona montana) fruit extract has never been conducted yet. Based on this reason, the research of secondary metabolite determination on soursop (Annona montana) fruit extract using the phytochemical screening to determine the secondary metabolite and antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method is necessary.

2. Research Method

2.1 Tools and Materials

The tools used were glassware, analytical balance, dropping pipette, test tube, test tube clamp, brown bottle, Buchner funnel, filter paper, aluminum foil, spectrophotometry UV-Vis, cuvette, and evaporator. The materials used were soursop (Annona montana) fruit, ethanol 70%, ethanol pa, Aquadest, concentrated HCl, HCl 2N, chloroform, Mg powder, Mayer's, Wagner's, Dragendorff's, and Lieberman-Bouchard's reagents, hot water, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) crystal.

2.2 Research Procedures

2.2.1 Plant Determination

The plant determination in this research was conducted by observing the soursop plant morphology, then matching it with the key determination in the Flora of Java book, supported by the data taken from the Plant Conservation Institution of Purwodadi Botanical Garden.

2.2.2 Extraction with the Maceration Method

The extraction of soursop fruit using the maceration method with the ethanol 70% dissolvent for 5x24 hours with the ratio of 1:7.5 was conducted. Then, the dissolvent was removed using the evaporator, continued by evaporating the dissolvent with the waterbath. The concentrated extract was gained. It was count for the yield. Also, the phytochemical testing of alkaloid, flavonoid, terpenoid, and saponin testing was conducted qualitatively.

2.2.3 Antioxidant Testing

The antioxidant testing using DPPH (2,2-diphenyl-1-picrylhydrazyl) with the DPPH solution concentration of 100.9 was conducted. For the primary solution, the DPPH solution concentration of 1162 ppm was used. Then, the dilution was conducted on the solution of 60, 70, 80, 90, 100 ppm in order. All the solutions were measured using the spectrophotometry UV-Vis with 515 nm of wavelength to obtain the absorbance value, continued by counting the % inhibition. Also, The IC_{50} value was obtained from the linear regression.

3. Results and Discussion

The plant determination, which was conducted by the Plant Conservation Institution of Purwodadi Botanical Garden, stated that the testing sample of soursop fruit from Jalan Barito 6, Bunulrejo, Blimbing, Malang, East Java is classified as Annonaceae family, Annona montana

Macfad species. The yield obtained from 300 grams of fresh soursop fruit was extracted with 2.250 liters of ethanol 70% for 5 days, then followed by the evaporation and waterbath process. From the concentrated extract of 25.5963 grams, the yield percentage is 8.3%.

3.1 Phytochemical Testing

From the extraction process, the concentrated extract was obtained. The concentrated extract was used in the qualitative phytochemical testing that resulted on the secondary metabolite. The secondary metabolites were flavonoid, alkaloid, saponin, and terpenoid. The phytochemical testing results can be seen in Table 1.

Table 1 Results of Phytochemical Testing

Sample	Secondary Metabolite	Reagent	Observation Result	Additional Information
Concentrated extract of soursop fruit	Flavonoid	Mg + HCl Concentrated Powder	Dark yellow solution	-
	Alkaloid	Mayer's	Light yellow solution without white sediment	-
		Wagner's	Reddish brown solution without light brown sediment	-
		Dragendorff's	Orange solution without brown sediment	-
	Terpenoid	Liebermen-Bouchard's	Brown ring was formed	+
	Saponin	Hot water + HCl 2N	No foam was formed	-

Note: (+) = exist; (-) = did not exist

Based on the table, in the flavonoid, alkaloid, and saponin testing demonstrated negative results, which means that the soursop fruit extract containing none of the compounds. On the contrary, the positive result was demonstrated by the terpenoid secondary metabolite. In the terpenoid testing, there was a change in color as mentioned in the table due to the oxidation of terpenoid through the double bond conjugated formation. The principal of reaction on the terpenoid testing reaction mechanism is condensation or the H_2O release and carbocation incorporation. This reaction is started with the acetylation of hydroxyl groups process using the acetic acid anhydride. The acetyl groups, which are the good go groups will release, forms double bonds. Next, the hydrogen groups and their electrons release occur causing the double bond movement. The terpenoid experiences a resonance acting as the electrophile or carbocation. The carbocation attack causes the electrophilic addition, followed by the hydrogen release. Then, the hydrogen groups and their electrons are released causing the terpenoid experiencing the conjugation extension forming a brown ring (Siadi, 2012). The antioxidant potential compound is terpenoid. Plants generally contain various kinds of free radical capture molecules like phenols (phenolic acid, flavonoid, quinone, coumarin, lignin, tannin, alkaloid, betalain, and terpenoid) that are rich in the antioxidant activity (Larson, 1988). Terpenoid is a phenol contains in plants (Harborne, 1987). Phenols can be a key determinant of food antioxidant potency (Parr & Bolwell, 2000). Since in phenols there is an aromatic ring, in terpenoid there is also the

aromatic ring. In phenols, there is C_6 structure, whereas, in terpenoid, there is only C_5 structure. All terpenoids come from the CH_2=C(CH_3)–CH=CH_2 isoprene molecule and their carbon framework are formed by the joining of two or more C_5 units (Harborne, 1987).

3.2 DPPH Testing

The antioxidant activity testing was conducted using DPPH method. According to some literature, the maximum wavelengths for DPPH are 515, 517, 518, 519, and 520 nm (Molyneux, 2004). In this research, the determination of DPPH wavelength with the concentration of 100 ppm was conducted on 515 nm. So, the form solution, which was DPPH, and sample solution were measured their absorbance at 515 nm of wavelength. On 515 nm of wavelength, the DPPH absorbance of 0.610 was obtained. The absorbance value was still in the range of 0.2 – 0.8. The ray absorbance range of 0.2 – 0.8 was selected as the sample orientation limit since, in the range, the resulted data were relatively linear (Ramadhan, 2013).

3.3 Antioxidant Activity Testing

The antioxidant activity testing using the spectrophotometer UV-Vis and the IC_{50} value calculation using the linear regression can be seen in Table 2.

Table 2 Result of Antioxidant Activity Testing of Soursop (*Annona montana*) Fruit Extract

Replication of	Sample	Concentration (ppm)	Absorbance		% Inhibition	Linear Equation	IC_{50}
			Form	Sample			
1	Ethanol Extract of Soursop	60	0.610	0.227	62.787	y = 0.109x + 55.96 R^2 = 0.947	54.67
		70		0.225	63.115		
		80		0.212	65.246		
		90		0.210	65.574		
		100		0.201	67.049		
2	Ethanol Extract of Soursop	60	0.610	0.226	62.951	y = 0.103x + 56.52 R^2 = 0.966	63.30
		70		0.224	63.278		
		80		0.213	65.081		
		90		0.209	65.737		
		100		0.202	66.885		
3	Ethanol Extract of Soursop	60	0.610	0.225	63.114	y = 0.101x + 56.85 R^2 = 0.953	67.82
		70		0.223	63.442		
		80		0.211	65.409		
		90		0.207	66.065		
		100		0.202	66.885		

In Table 2, the absorbance difference and % inhibition of each sample concentration (ppm) can be understood. The absorbance value depends on the substance level contained inside it. The higher substance level contained inside a sample, the more molecules absorbing the light on the certain wavelength. So, the absorbance value is also higher or in other words, the absorbance value is directly proportional to the substance concentration inside a sample (Neldawati et al., 2013). Therefore, in determining the IC_{50} value, the analysis using the linear regression is necessary. After three times of replication, the IC_{50} value for each replication was obtained and presented in Table 3.

Table 3 IC_{50} Value for Each Replication and IC_{50} Average Value

Replication of	IC_{50} Value (ppm)
1	54.67
2	63.30
3	67.82
Average	61.93

In Table 3, the first replication of soursop (Annona montana) fruit extract resulted in the IC_{50} value of 54.67. The second replication resulted in the IC_{50} value of 63.30, whereas on the third replication, the IC_{50} value of 67.82 was obtained. From the results of each replication, the IC_{50} average value of 61.93 was obtained. The value can be categorized as a strong antioxidant since according to the standard, the IC_{50} value of 50 – 100 is included as a strong antioxidant. The IC_{50} value can be categorized into 4. If the IC_{50} value < 50, the antioxidant activity is very strong; if the IC_{50} value = 50 – 100, the antioxidant activity is strong; if the IC_{50} value = 100 – 150, the antioxidant activity is average; and if the IC_{50} value = 150 – 200, the antioxidant activity is weak (Molyneux, 2004). So, the soursop fruit extract (Annona montana) has a potency as an antioxidant although its antioxidant activity is lower than the vitamin C antioxidant activity. Putri (2012) found that vitamin C has a very strong antioxidant with the IC_{50} value of 3.72 ppm. Prasetyorini et al. (2014) also found that vitamin C has a very strong antioxidant activity of 4.15 ppm. On the contrary, it is found that soursop (Annona muricata) fruit extract has a weak antioxidant activity of 282.61 ppm. Yet, in this research, it is found that soursop (Annona montana) fruit extract has a strong antioxidant activity with the IC_{50} average value of 61.93 ppm.

4. Conclusion

Based on the research, it is found that the soursop (Annona montana) positively containing terpenoid secondary metabolite and antioxidant activity. The soursop fruit extract has the IC_{50} average value of 61.93 from three times of replication. It is categorized as a strong antioxidant.

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