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Antioxidant Activity and Bioactivity (LC_{50}) of Soursop Leaves Jelly Candy with Addition of Soursop Fruit Extract (*Annona muricata* L)

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Abstract: It has been reported that the leaves of the soursop contains acetogenin that have the potential to inhibit the growth of cancer cells. Soursop leaves has not been used optimally, nowadays its leaves have only processed into tea or in capsules for drug treatment and also just to keep the body stamina. In this study had been tried to make Jelly Candy from the leaves, but there are weaknesses in flavor and taste. This study used a completely randomized design (CRD) consist of 5 treatments and 3 replications. Data were analyzed statistically used ANOVA and followed by Duncan's New Multiple Range Test (DNMRT) at 5% significance level, the treatment were addition of soursop fruit extract as follows: A (0%), B (10%), C (20%), D (30%) and E (40%). Antioxidant activity was analyzed using DPPH method and bioactivity using Brine shrime Lethality Test (LC_{50}). The results showed that the addition of the soursop fruit extract had significant effect on the antioxidants activity and bioactivity in the product was weak (LC_{50}>1000 µg/mL).

Key words: Antioxidant activity, bioactivity, fruit soursop, jelly candy, leaves soursop

INTRODUCTION

Rieser *et al.* (1993) showed that the soursop leaves has a group of compounds *acetogenins*. *Annonaceous acetogenins* from *Annona muricata* L found to be anti-tumor and anti-cancer agent as it had recently done in many *in vitro* studies. These acetogenins showed a selective way to fight various types of cancer cells without damaging healthy cells.

Soursop leaves only processed into tea or capsules for medication and also just to maintain stamina. Therefore, it is necessary to create an innovative product from the leaves of the soursop. One of the products that can be produced by using soursop leaves as raw material is jelly candy. The jelly candy was weakness in flavour and taste.

Soursop fruits known to have a strong smell and flavour, because of the high content of vitamin C, but it also contained vitamin A and B complex. Mineral content quite complete coupled with amino acids such as lysine, methionine and triptopan (United State Department of Agriculture Human Nutrition Information Service, 1982). However fresh soursop fruit was not favoured by many people, because of the taste that sour. Ripe soursop fruit has a soft texture. Ripe soursop fruit also had a short shelf life, so it will reduce the quality and price of soursop fruit in the market. Therefore, the addition of the soursop fruit on soursop jelly candy not only to improve the taste, but also as a source of vitamins, minerals and amino acids that were not given from the soursop leaves and also expected to improve the utilization of soursop fruits.

This study aimed to determine antioxidant activity and bioactivity (LC_{50}) of soursop leaves jelly candies with the addition of soursop fruit extract (*Annona muricata*, L).

MATERIALS AND METHODS

Research design: The design used completely randomized design (CRD) with 5 treatments and 3 replications. Data were analyzed statistically used ANOVA and if it significantly different then followed by Duncan's test New Multiple Range Test (DNMRT) at the 5% significance level. Treatment was addition of soursop fruit extract as follows: A (0%), B (10%), C (20%), D (30%) and E (40%).

Making of soursop leaves jelly candy: Making of jelly candy begins with making of soursop leaves extract and soursop fruit extract. Soursop leaves extract made from 10 sheets of soursop leaves were washed and reduced in size ±1 cm added with 300 ml of water and then boiled until the remaining 100 ml. To get soursop fruit extract done by used an extractor and then filtered with gauze.

A total of 100 ml of soursop leaves extract added with liquid palm sugar and glucose syrup. Then heated until dissolved, stirred frequently. Then added the gelatin and stirred until dissolved. The mixture was cooked, stirred constantly until thickened (±20 min). Soursop extract then inserted according to treatment (0, 10, 20, 30 and 40%) and then cooked with a maximum temperature of 40°C while stirring until cooked signed with no parts fall when it taken with a fork. Then lifted and molded candy

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and left for 1 h at room temperature. Chill in refrigerator for 24 h. After that took it from the refrigerator and let it at room temperature for 1 h and remove it from the mold, cut into a square (±1.5 x 1.5 x 1 cm) coated with flour and sugar and then packaged.

Observation: The observations in this study were antioxidant activity, using DPPH Method (Huang *et al*., 2005); vitamin C, using Titrimetric Method (Sudarmadji *et al*., 1997); pH, using pH meter (AOAC, 1995); bioactivity, using Brine Shrimps Lethality Test (LC_{50}; Fero, 2006); moisture content using a moisture analyzer KERN DLB; ash content (Sudarmadji *et al*., 1997); aw using a series labmaster aw; reducing sugar using titrimetric method (BSN, 2008); sucrose using titrimetric method (BSN, 2008) on the raw material and the jelly candies.

RESULTS AND DISCUSSION

Raw material: Vitamin C, pH, antioxidant activity and cytotoxic test (LC_{50}) of the raw materials showed in Table 1. There is no vitamin C in the leaves extract, but in the fruit extract (19.52 mg/100 g). Vitamin C is one of factors that effect on antioxidant activity. Although there is no vitamin C in the leaf, but antioxidant activity in leaves extract was higher (57.515) than the fruit extract (38.73%). This indicates that there is another component in the leaves extract that effect on the antioxidant activity.

Latifah (2013) reported that antioxidant activity in green leaves extract (ethanolic extract) with an IC_{50} value was 141.00 ppm (w/v). IC_{50} value less than 200 ppm showed it had a strong antioxidant activity. It was related with the flavonoid compound like flavones, dihidroflavonol, flavonol and flavanones. Further explained that flavonol was the most active.

LC_{50} value of the soursop leaves extract and soursop fruit extract were 299.92 and 1142.87 µg/mL, respectively. This reveals that a soursop leaves extract contain active component. According to Meyer *et al*. (1982), that a compound said to be active if it has LC_{50} values below 1000 µg/mL.

LC_{50} values indicate a presence of active component that are cytotoxic. The active compound is annonaceous acetogenins that toxic to cancer cells. Geum-Soog *et al*. (1998) said Annonaceous acetogenins showed strong biological activities, such as *in vivo* antitumor, cytotoxic, pesticides, antibacterial effect and antiparasitic.

Characteristics of jelly candy: Table 2 showed that the texture, moisture content, ash content, reducing sugar, sucrose of different treatment were different significantly at α = 5%, but the aw of different treatment was not different significantly at α = 5%. The texture of the jelly candy was influenced by the addition of fruit extract. The higher of fruit extract added the higher of hardness. The range of texture was 0.76 until 1.33 mJ. This data shown that the hardness value was inversely related to the addition of fruit extract, the more addition of fruit extract, the lower hardness value.

Fig. 1: Jelly Candy made from different addition of Soursop fruit extract

Table 1: Characteristics of soursop leaves and soursop fruit extract

	Soursop leaves extract	Soursop fruit extract
Vitamin C (mg/100 g)	Not detected	19.52
pH	6.10	3.30
Antioxidant activity (%)	57.51	38.73
LC_{50} (µg/mL)	299.92	1142.87

The range of moisture content was 17.75 until 19.76%. The higher fruit extracts added the higher moisture content level. It was due to moisture content of the fruit extract was higher. According to Kusnandar (2010), the water in the food found between the cells, trapped in cells or bound to a chemical compound in the food. All of soursop leaves jelly candy were still eligible to SNI for jelly candy that was 20%.

The fruit extract addition was also increase ash content of jelly candy. Ash content shows that element content in the jelly candy. Increased levels of ash in the jelly candy in line with the increase of fruit extract. This is related to mineral content in the soursop fruit. According to the United State Department of Agriculture Human Nutrition Information Service (1982), soursop fruit contains a 14.00 mg of calcium, 0.60 mg of iron, 21.00 mg of magnesium, 27.00 mg of phosphorus, 14.00 mg of potassium and 278.00 mg of sodium. Winarno (1997), ash content describe minerals content in food. All of the jelly candy were eligible to SNI, that was 3% maximum.

The value of the reducing sugar of jelly candy increased along with the increasing of fruit extract added. It was caused of the fruit naturally contain reducing sugars such as fructose; so that the higher of fruit extract added, the higher the level of reducing sugars in the jelly candy. All of the jelly candy have reducing sugar level eligible to SNI, that is 25% maximum.

The more addition of fruit extract the higher the sucrose level of the jelly candy. It was caused the more addition

Table 2: Characteristic of jelly candy made from different treatment

Addition of soursop fruit extract	A (0%)	B (10%)	C (20%)	D (30%)	E (40%)	p
Texture (mJ)	1.33±0.02	1.25±0.01	1.07±0.02	0.97±0.02	0.76±0.01	<0.05
Moisture content (%)	17.75±0.94	18.43±0.48	18.85±0.07	19.36±0.17	19.76±0.11	<0.05
Ash content (%)	0.82±0.02	0.86±0.04	0.89±0.44	0.93±0.06	0.95±0.01	<0.05
Reducing sugar (%)	1.45±0.01	1.94±0.01	2.96±0.01	5.95±0.13	6.01±0.01	<0.05
Sucrose (%)	15.34±0.03	15.56±0.13	16.60±0.23	20.44±0.20	20.65±0.10	<0.05
Aw	0.80±0.002	0.80±0.005	0.79±0.004	0.79±0.001	0.79±0.003	>0.05

Table 3: Vitamin C and pH of jelly candy made from different treatment

Addition of soursop fruit extract	Vitamin C (mg/100 g)	pH
A (0%)	0.00±0.00^{e}	6.08±0.085^{a}
B (10%)	3.92±0.104^{d}	5.92±0.070^{b}
C (20%)	4.62±0.032^{c}	5.84±0.061^{bc}
D (30%)	5.62±0.059^{b}	5.77±0.026^{cd}
E (40%)	6.06±0.180^{a}	5.71±0.025^{e}

Numbers in the same column followed by the same lowercase letter are not significantly different according to DNMRT at 5% significance level

Table 4: Results of analysis of antioxidants activity and LC_{50} of soursop leaves jelly candy

Addition of soursop fruit extract	Antioxidants activity (%)
A (0%)	8.09±1.9509^{e}
B (10%)	18.30±0.0425^{d}
C (20%)	25.96±0.0000^{c}
D (30%)	45.53±0.0000^{b}
E (40%)	55.32±0.0425^{a}

Numbers in the same column followed by the same lowercase letter are not significantly different according to DNMRT at 5% significance level

of fruit extract increased the water content of the jelly candy. Increasing water content of the jelly candy, it needed more sugar used for coating until saturated, so the more sucrose was in the jelly candy. The range of aw of the jelly candy was 0.79 until 0.80 and there is not different significantly at α = 5%.

Vitamin C and ph of jelly candy: Vitamin C and pH of Jelly Candy that was made from different treatment shown in Table 3.

Vitamin C: The results of analysis of vitamin C in the jelly candies shown in Table 3. The vitamin C content of this candy is affected by increasing number of soursop extract added to the formulation. It is related with vitamin C fruit extract that was 19.52 mg/100 g. This values almost the same with another report. Persatuan Ahli Gizi Indonesia (2009) reported that Soursop fruit has vitamin C content as 20 mg/100 g, thus addition of the fruit extract increasing the levels of vitamin C in the soursop leaves jelly candy.

pH: The results of the analysis on the pH value of the jelly candies shown in table 3, the highest of pH value of jelly candy is treatment A (addition of soursop extract 0%) that was 6.08 and the lowest pH values was

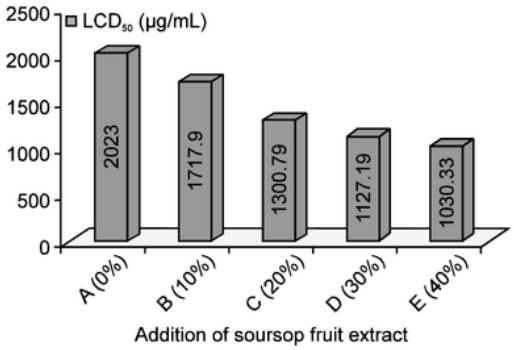

Fig. 2: Bioactivity of Jelly Candies made from different addition of Soursop fruit extract

treatment E (addition of soursop extract 40%) that was 5.71. The pH of jelly candy decreased along with the addition of fruit extract. The soursop fruit extract rich vitamin C that lower the pH.

Antioxidant activity: Table 4 showed that the addition of fruit extract increase the antioxidant activity significantly. In a preliminary analysis of soursop leaves extract known that it had antioxidant activity as much as 57.51% and antioxidant activity of soursop fruit extract as much as 38.73%. After mixing both of this material and was processed by using heat, it make decreased the activity. The higher addition of fruit extract, the higher activity. It is assumed that the heat treatment will decrease the activity resulting from the active component in extract of soursop. According to Kardinan (2004), soursop leaves contain active ingredients annonain, saponins, flavonoids, tannins. According to Emam *et al*. (2009) Flavonoids is known as one of the powerful antioxidant substances are very strong that it can eliminate the damaging effects of oxygen which occurs in the human body.

Antioxidant activity in the jelly candy is more influenced by active component in the fruit extract. This is presumably because the extraction process that has been used. The leaf extract obtained by boil the leaves, while the soursop fruit extracts obtained through the process of destruction of the fruit without the use of heat. So that the active compounds in fruit is slightly damaged. Active compounds are compounds that can be damaged by heat treatment. It was assumed that loss of active

compounds is occur on the process of raw material extraction, but in the process of making jelly, the active compounds losses is very small due to the temperature used is 60°C. The addition of fruit extract increased the antioxidant activity of jelly candy. The addition of soursop fruit extract increased the antioxidant activity of jelly candy.

Bioactivity (LC_{50}): The results of the analysis of BST (Brine Shrimp Lethality Test) as shown in Fig. 2, the lowest LC_{50} value was jelly candy with treatment E (addition of soursop fruit extract 40%) with LC_{50} value as much as 1030.33 µg/mL. Analysis of the bioactivity of the leaf has LC_{50} values as much as 299.92 µg/mL. While the soursop extracts LC_{50} value is equal to 1142.87 µg/µmL. Based on analysis of the bioactivity of these jelly candy can be said is off because it has LC_{50} values> 1000 µg/mL. This is in accordance with the opinion of Meyer *et al.* (1982) who found that the compound said to be active if it had LC_{50} values below 1000 µg/mL.

Based on Fig. 2 showed that the higher fruit extract added in the formula, the higher of bioactivity of the jelly candy. It assumes that there is a compound in the fruit extract has toxic activity. From the raw analysis, it was found that fruit extract rich in vitamin C, so maybe it was related with the vitamin C.

If it was compared between the raw material with the jelly candies produced, the bioactivity of those are very different. It is assumed due to the use of heat, in which two-time warming on leaf extraction. First when making extracts and second when making jelly candy that use temperature 60°C. Allegedly it can cause damage acetogenins compounds in the raw materials. The other reason is the lower Soursop leaf concentration in the formulation, because of the addition of fruit extract and other component.

Conclusions: The addition of Soursop fruit extract has a significant effect on the texture, moisture content ash content, reducing sugar, sucrose, of the Jelly Candy, but has no significant effect on the aw of the jelly candy. The addition of Soursop fruit extract has a significant effect on the pH value, vitamin C and antioxidant activity of the Jelly Candies. The higher concentration of fruit extract added, the higher the vitamin C, the lower the pH, the higher the antioxidant activity and the higher bioactivity. The bioactivity of all product is weak (LC_{50}>1000 µg/mL).

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