

Study Of The Antibacterial Activities Of Soursop (*Annona muricata* L.) Leaves

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Abstract : Background: The use of natural product medicines has emerged from traditional to modern therapy in order to increase the quality of health worldwide. Nature-derived medicines are considered safer. The leaves of soursop (*Annona muricata* L.) has long been used by certain local communities in Indonesia as an alternative treatment of bacterial diseases.

Objective: Objective of this study was the investigation of antibacterial activities of methanol extract and chloroform fraction of the leaves of soursop (*Annona muricata* L.) of the family of Annonaceae against *Escherichia coli* and *Staphylococcus aureus*

Methods: The methanol extract and chloroform fraction were obtained by means of maceration method using methanol as solvent and then fractionated, with chloroform. The antibacterial activities were measured *in vitro* by means of agar diffusion method using paperdisc.

Results: Methanol extract of soursop leaves at concentration of 150 mg/ml could inhibit the growth of *Staphylococcus aureus* with inhibition zone diameter of 14.1 mm and of 13.1 for *Escherichia coli*. But the methanol extract of soursop leaves at concentration of 250 mg/ml could inhibit the growth of *Escherichia coli* with inhibition zone diameter of 14.5 mm. It fulfilled the requirement of Farmakope Indonesia. Chloroform fraction of soursop leaves at concentration of 150 mg/ml could only inhibit the growth of *Staphylococcus aureus* with inhibition zone diameter of 9.9 mm and of 9.2 mm for *Escherichia coli*.

Conclusion: The methanol extract indicated a higher inhibition zone diameter than chloroform fraction against *Staphylococcus aureus* and *Escherichia coli*.

Key words: soursop leaves, antibacterial activities, *Escherichia coli* and *Staphylococcus aureus*.

Introduction

We previously have reported our investigation about the characterization and the phytochemical screening of the leaves of soursop (*Annona muricata* L.). The alkaloids, flavonoids, tannins, saponins, glycosides and steroid/triterpenoids were present on the leaves. The antibacterial activities against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acne* and *Pseudomonas aeruginosa* were also positively found¹.

Soursop (*Annona muricata* L.) is a tropical plant and familiar to the Indonesian certain local communities. This plant has great benefits for human life which is full with nutrition. In the food industry soursop can be

processed into jam, fruit juice, syrup². Soursop leaves contain flavonoid, tannin, alkaloid, saponin, calcium, phosphor, carbohydrate, vitamin A, B and C, phytosterol, calcium oxalate³.

These leaves are traditionally used to prevent and treat arthritis, asthma, bronchitis biliary disorder, diabetic, heart diseases, hypertension, worm disease, liver disorder, malaria, rheumatism, sedative, tumor, and cancer⁴. The leaves are also used for the treatment of several types of diseases caused by bacteria such as pneumonia, diarrhea, urinary tract infection, and other kinds of skin diseases⁵.

Staphylococcus aureus is a gram positive bacteria that is commonly found on human skin and mucous membranes. This bacteria can cause skin infection⁶. *Escherichia coli* is a gram negative bacteria that is commonly found in the human colon. This bacteria is one of the most common pathogenic bacteria in food causing the primary infection of the intestine such as diarrhea⁷.

Based on those facts, in order to continue our investigation we report here our study about the antibacterial activities of soursop (*Annona muricata* L.) leaves against *Escherichia coli* and *Staphylococcus aureus*.

Experimental Methods

Materials

Materials used in this study were soursop (daun sirsak, *Annona muricata* L.) leaves, agar nutrient media, broth nutrient media, *Staphylococcus aureus* (ATCC No. 6538) and *Escherichia coli* (ATCC No. 25922), and aquadest.

Apparatus

Apparatus used in this study were glasses, autoclave (Fisons), blender (Philips), freeze dryer (Modulio), incubator (Fiber Scientific), filter paper, Laminar Air Flow Cabinet (Astec HLF 1200L), refrigerator (Toshiba), oven (Mettler), heater, micro pipet (Eppendorf), rotary evaporator (Haake D), visible spectrophotometer (Dynamic), digital balance (Mettler Toledo).

The Collection and The Treatment of Soursop Leaves

The leaves of soursop (*Annona muricata* L.) were purposively collected from Kelurahan Tanjung Mulia, Kecamatan Medan Deli, Medan city, Indonesia in January 2013. The fourth and the fifth leaves were picked out of the point of a young leaf.

The Identification of Soursop Leaves

It was conducted by the Herbarium Medanense, University of Sumatera Utara.

The Preparation of Symplex

The leaves of soursop was weighed after washing and drying in open air. They were blended to obtain a powder mass and kept in a closed pocket of plastics.

The Preparation of Extract of Soursop (*Annona muricata* L.) Leaves

500 g of symplex powder of soursop leaves were macerated for 5 days out of light, occasionally stirred, by maceration method using methanol as solvent. After 5 days, the mixture was filtered and the residue washed by using methanol and treated for 2 days of the same treatment as before. The macerates were then mixed together and concentrated by means of rotary evaporator with the temperature not exceeding 40°C until obtained spissum extract by means of freeze dryer⁸.

The Preparation of Media

Agar nutrient media

Composition: Lab-lemco powder	1 g
Yeast extract	2g
Peptone	5 g
Sodium chloride	5 g
Agar	15 g

The preparation⁹: 28 g of agar nutrient media was suspended in 1000 ml of distilled water and heated to completely dissolved. The media was then put into flask and sterilized for 15 minutes at 121°C.

Broth nutrient media

Composition: Lab-lemco powder	1 g
Yeast extract	2 g
Peptone	5 g
Sodium chloride	5 g

The preparation⁹ : 13 g of broth nutrient media was suspended in 1000 ml of distilled water and heated to completely dissolved. The media was then put into flask and sterilized for 15 minutes at 121°C.

The Sterilization of Apparatus

The glasses used in this antibacterial activities test was sterilized in oven for 1 hour at the temperature of 170°C. The media was sterilized in autoclave for 15 minutes at 121°C. Alat-alat yang digunakan dalam uji aktivitas antibakteri ini, disterilkan terlebih dahulu sebelum dipakai. The ose syringe and pincet were sterilized by means of Bunsen lamp¹⁰.

The Preparation of Chloroform Fraction of Soursop (*Annona muricata* L.) Leaves

10 ml of methanol solvent was added into 10 g of methanol extract, then homogenously stirred and removed into separating funnel and 20 ml of distilled water and 40 ml of *n*-hexane were added, stirred and let it separated and fractionated completely by *n*-hexane, the residue of methanol extract was then fractionated by 40 ml of chloroform. The result of chloroform fractionation was then evaporated on a waterbath to obtain a dry extract of chloroform fraction.

The Preparation of Bacteria Culture Stock¹¹

The colony of bacteria was taken by sterilized ose syringe, then planted into sloping agar nutrient media by scratching it. It was then incubated in a incubator for 18 – 24 hours at the temperature of 36-37°C.

The Preparation of Bacteria Inoculum

The colony of bacteria was taken from culture stock by sterilized ose syringe, then suspended into test tube of broth nutrient media of 10 ml. The turbidity of the solution was then measured at the wavelength of 580 nm untill obtained the transmittance of 25% equivalent with 10^6 CFU (Colony Forming Units)¹¹.

The Preparation of Solution Tests of Methanol Extract and Chloroform Fraction with Various Concentrations

3 g of methanol extract was dissolved into DMSO untill 10 ml of volume in order to obtain the concentration of the extract of 300 mg/ml. The dilution was then made in order to obtain extracts with the concentrations of 250 mg/ml; 200 mg/ml; 150 mg/ml; 100 mg/ml; 50 mg/ml; 25 mg/ml; 10 mg/ml; 5 mg/ml. The same procedure was done to chloroform fraction.

The *In Vitro* Antibacterial Activities Tests

0.1 ml of inoculum was put into petridisc was then added 20 ml of sterillized liquid agar nutrient media heated gently to 45°C, homogenized and let it stand in order to obtain a solid media. The paper disc with diameter of 6 mm was soaked into solution tests of various concentrations, dried and let them on the surface of agar nutrient media. It was then incubated for 18-24 hours at the temperature of 36-37°C. The same procedure was done to chloroform fraction. The inhibition zone diameter around the paperdisc was then measured and recorded by what so called *jangka sorong*. The tests were respectively done 3 times¹¹. The tests of antibacterial activities of methanol extract and chloroform fraction of soursop (*Annona muricata* L.) leaves against *Staphylococcus aureus* and *Escherichia coli* are depicted in the next Figure 1, 2, 3 and 4.

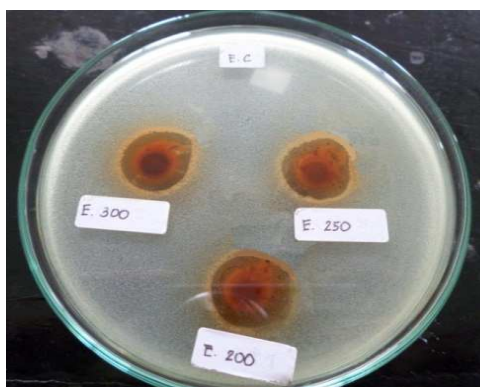


Fig. 1. The test of antibacterial activities of methanol extract of soursop (*Annona muricata* L.) leaves against *Staphylococcus aureus*

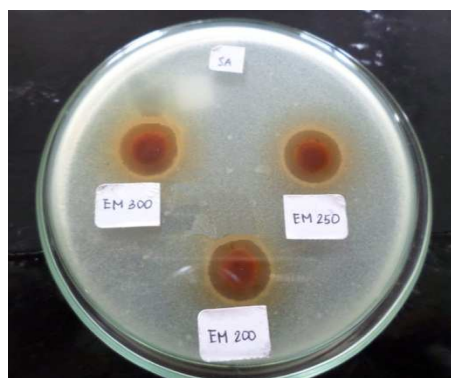


Fig 2. The test of antibacterial activities of methanol extract of soursop (*Annona muricata* L.) leaves against *Escherichia coli*

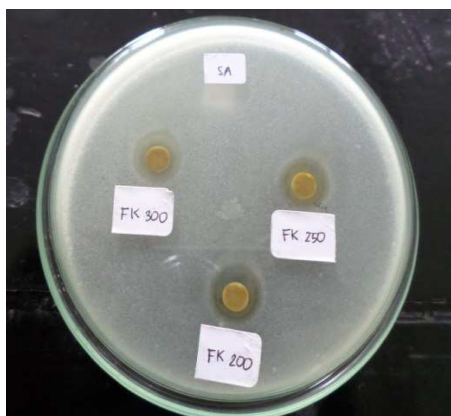


Fig. 3. The test of antibacterial activities of chloroform fraction of soursop (*Annona muricata* L.) leaves against *Staphylococcus aureus*

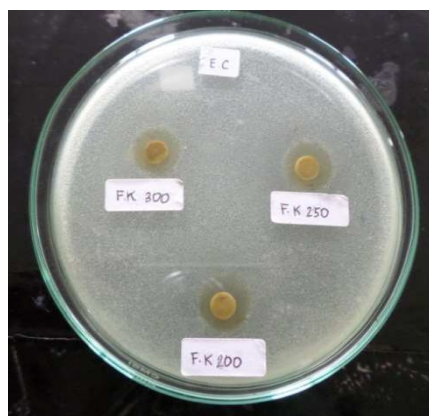


Fig. 3. The test of antibacterial activities of chloroform fraction of soursop (*Annona muricata* L.) leaves against *Escherichia coli*

Results And Discussion

The antibacterial activities test was conducted in various concentrations to investigate their relationship. The results of antibacterial activities examination showed that methanol extract and chloroform fraction gave antibacterial effect against *Escherichia coli* and *Staphylococcus aureus*. They were denoted by the existence of inhibition zone around the paperdisc. The results of the average of inhibition zone diameter of methanol extract and chloroform fractions of soursop leaves for *Staphylococcus aureus* and *Escherichia coli* can completely be seen on Tabel 1 and Tabel 2 below.

Tabel 1. The results of the average of inhibition zone diameter of the growth of *Staphylococcus aureus* and *Escherichia coli* of methanol extract of soursop leaves

Methanol extract concentration (mg/ml)	Inhibition Zone Diameter (mm)*	
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
300	16.4	15.3
250	15.4	14.5
200	14.7	13.8
150	14.1	13.1
100	13.1	12.3
50	12.2	11.5
25	11.2	10.7
10	9.8	8.7
5	8.6	8.0
Blank	-	-

Informations: (*) = The average results of triple measurements, (-) = no inhibition

On the Tabel 1 above it is obvious that the increase of extract concentrations will enhance their antibacterial activities caused by the increase of bioactive contents of the extract.

The results of antibacterial activities examination showed that methanol extract at concentration of 150 mg/ml could inhibit the growth of *Staphylococcus aureus* with inhibition zone diameter of 14.1 mm and at concentration of 250 mg/ml could inhibit the growth of *Escherichia coli* with inhibition zone diameter of 14.5 mm. The Minimum Inhibition Concentration (MIC) of methanol extract at concentration of 5 mg/ml could inhibit *Staphylococcus aureus* with inhibition zone diameter of 8.6 mm and of 8.0 mm for *Escherichia coli*.

The inhibition zone diameter of bacterial growth of methanol extract of soursop was larger than of ethanol extract¹ caused by the strength of methanol compared with ethanol as a solvent of bioactive compound¹².

Tabel 2. The results of the average of inhibition zone diameter of the growth of *Staphylococcus aureus* and *Escherichia coli* of chloroform fractions of soursop leaves

Chloroform fractions concentration (mg/ml)	Inhibition Zone Diameter (mm)*	
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
300	12.1	11.5
250	11.5	10.7
200	10.5	9.8
150	9.9	9.2
100	9.3	8.8
50	8.8	8.3
25	-	-
10	-	-
5	-	-
Blank	-	-

Informations: (*) = The average results of triple measurements, (-) = no inhibition

The chloroform fractions at concentration of 300 mg/ml showed the inhibition zone diameter of 12.1 mm for *Staphylococcus aureus* and of 11.5 mm for *Escherichia coli*. The Minimum Inhibition Concentration (MIC) at concentration of 50 mg/ml showed the inhibition zone diameter of 8.8 mm for *Staphylococcus aureus* and of 8.3 mm for *Escherichia coli*.

A compound with inhibition zone diameter about 14 through 16 mm is said to have a satisfied inhibition zone diameter¹¹. The inhibition zone diameter of methanol extract fulfilled the requirement. It could be understood,

maybe because of methanol extract containing tannin and flavonoids, whereas chloroform fraction containing only flavonoids in small amount. The mechanism of tannin with antibacterial activities on low concentration was by destroying the cytoplasm membrane causing the leak of cell, on high concentration the tannin would coagulate with cellular protein¹³. The flavonoids could damage the permeability of cell wall of bacteria¹⁴.

The result of this study indicated that *Staphylococcus aureus* as positive gram bacteria is more sensitive to chemicals than *Escherichia coli* as negative gram bacteria. This thing could be caused by the difference in composition and structure of cell wall of those bacteria. The structure of cell wall of positive gram bacteria consists of lipid only (1 – 4 %), whereas of negative gram bacteria consists of multi-layers of lipoprotein, liposaccharide and peptidoglycan with a high lipid content (11 – 12 %).

Conclusion

This study indicated that methanol extract of soursop leaves at concentration of 300 mg/ml could inhibit the growth of *Staphylococcus aureus* with inhibition zone diameter of 16.4 mm and of 15.3 mm for *Escherichia coli*. Chloroform fraction of soursop leaves at concentration of 300 mg/ml could only inhibit the growth of *Staphylococcus aureus* with inhibition zone diameter of 12.1 mm and of 11.5 mm for *Escherichia coli*.

Methanol extract of soursop leaves at concentration of 150 mg/ml could inhibit the growth of *Staphylococcus aureus* with inhibition zone diameter of 14.1 mm and of 13.1 mm for *Escherichia coli*. But the methanol extract of soursop leaves at concentration of 250 mg/ml could inhibit the growth of *Escherichia coli* with inhibition zone diameter of 14.5 mm. It fulfilled the requirement of Farmakope Indonesia, they are of 14 through 16 mm. The minimum inhibitory concentration (MIC) of methanol extract at concentration of 5 mg/ml could inhibit the growth of *Staphylococcus aureus* with inhibition zone diameter of 8.6 mm and of 8.0 mm for *Escherichia coli* respectively. The MIC of chloroform fraction at concentration of 5 mg/ml did not inhibit growth of *Staphylococcus aureus* and *Escherichia coli* at all. Whereas the MIC of chloroform fraction at concentration of 50 mg/ml could only inhibit *Staphylococcus aureus* with inhibition zone diameter of 8.8 mm and of 8.3 mm for *Escherichia coli*.

The methanol extract indicated a more effective bacterial inhibition than chloroform fraction of the same concentration.

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