

## THE EFFECTIVENESS OF SOURSOP LEAF EXTRACT AGAINST GROWTH OF *AGGREGATIBACTER ACTINOMYCETEMCOMITANS* ATCC® 6514™ *IN VITRO*

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### ABSTRACT

**Objective:** The objective of this study was to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) antibacterial power of soursop leaf extract on *Aggregatibacter actinomycetemcomitans* (Aa) ATCC® 6514™ growth.

**Methods:** This study was experimental laboratory with post-test only control group design and consists of 8 treatment groups that were soursop leaf extract group with concentration 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.5625% as well as negative control groups were brain heart infusion broth (BHIB) media and chlorhexidine as positive controls. Each treatment was done 3 repetitions. Testing the effectiveness of soursop leaf extract using dilution methods on BHIB and subculture media on Mueller Hinton Agar (MHA) media. The number of Aa ATCC® 6514™ colonies was calculated manually using the total plate count method on the MHA media. Data were analyzed using Kruskal-Wallis test ( $p < 0.05$ ) followed by least significance different (LSD) test to see the significant mean difference between treatment groups.

**Results:** Concentration of MIC from soursop leaf extract on Aa ATCC® 6514™ growth was 1.5625% and MBC was 6.25%. LSD assay results showed significant difference effect ( $p < 0.05$ ) Aa ATCC® 6514™ from each treatment group.

**Conclusion:** Soursop leaf extract has antibacterial effectivity against Aa ATCC® 6514™.

**Keywords:** Soursop leaf extract, Antibacterial, *Aggregatibacter actinomycetemcomitans* ATCC® 6514™, Minimum inhibitory concentration, Minimum bactericidal concentration.

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### INTRODUCTION

Soursop plants (*Annona muricata* L.) are widely publicized as multicellular medicinal plants and often referred to as magic trees. Populations of various countries in the world have long-used soursop plants as herbal remedies. All parts of soursop plants can be used as herbal remedies such as bark, leaves, roots, fruit, and seeds. From all parts of the soursop plant, leaves are most often used to treat the disease [1].

Soursop leaf is a part that has a lot of chemical compounds that are very high active such as tannins, alkaloids, flavonoids, and terpenoids [2]. This plant showed the ability to inhibit the growth of cariogenic bacteria. Many active chemical compounds, especially terpenoids, are thought to have potential as an antibacterial, antidiabetic potentials, antihypertensive properties, antioxidative, and anticancer effects [3-6].

Ethanol extract of soursop leaf (*A. muricata* L.) had antibacterial activity against ATCC® 35668™ *Streptococcus mutans* with minimum inhibitory concentration (MIC) at the concentrations of 125 mg/mL [7]. Soursop leaf ethanolic extract has shown the highest antibacterial activity toward *Pseudomonas aeruginosa* and *Staphylococcus aureus* [8]. Soursop leaf extract can inhibit the growth of supragingival plaque bacteria with MIC at a concentration of 12.5% [9].

*Aggregatibacter actinomycetemcomitans* (Aa) are Gram-negative bacteria that have small, non-motile, capnophilic, fermentative coccobacillus form. These bacteria are commensal in the oral cavity but are often found in dental plaque, periodontal pocket, and gingival

sulcus. The role of these bacteria can cause various infections in humans such as endocarditis, brain abscesses, and periodontal disease [10].

Soursop leaf extract was shown to have antibacterial activity against mixed periodontal pathogen bacteria. Bacterial mixed periodontal pathogen is a term for bacteria that play a role in the pathogenesis of periodontal disease. One of the most dominant bacteria found is Aa. Examination of antibacterial activity of soursop leaf extract by measuring the inhibition zone of soursop leaf extract when mixed with pathogens so that the best concentration in inhibiting the bacteria was 45 mg/mL [11].

### Soursop leaf (Fig. 1)

#### Taxonomy

Kingdom: Plantae  
 Subkingdom: Tracheobionta  
 Superdivision: Spermatophyta  
 Division: Magnoliophyta  
 Class: Magnoliopsida  
 Subclass: Magnoliidae  
 Order: Magnoliales  
 Family: Annonaceae  
 Genus: Annona  
 Species: *Annona muricata* Linn.

### Morphology of soursop leaf

The leaves are elliptical with short pointed tip, measuring 8–16×3–7 cm. Leaf stalk length 3–7 mm, flat edges, and glossy leaf surface. The old



Fig. 1: Soursop leaf

leaves are dark green, while the young leaves are yellowish green. Soursop leaves are thick and somewhat stiff with pinnate leaf veins or erect in the main leaf veins.

#### Soursop leaf contents

Soursop leaf proved to have a lot of nutrients and minerals which are very useful for the body.

Some of them are acetogenins, annocatacin, annocatalin, annohexocin, annonacin, anomurian, annomurine, anonol, caclourine, gentisic acid, gigantertronin, linoleic acid, muricapentocin, niacin, phosphorus, calcium, carbohydrate, Vitamin C, Vitamin B<sub>1</sub>, Vitamin B<sub>2</sub>, and many nutritional contents; then, the benefits of soursop leaves are very good for health [12]. Soursop leaves also have antibacterial compounds such as steroids, saponins, tannins, and flavonoids [13].

#### *Aggregatibacter actinomycetemcomitans* (Fig. 2)

##### Taxonomy

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Pasteurellales

Family: Pasteurellaceae

Genus: *Aggregatibacter*

Species: *Aggregatibacter actinomycetemcomitans*

Aa may produce virulence factors that can improve its ability to persist in the oral cavity. These virulence factors are involved in the pathogenesis of periodontitis. These virulence factors include lipopolysaccharide (endotoxin), leukotoxin (forming a hole in granulocyte neutrophil, monocytes and some lymphocytes that consequently die from osmotic pressure), collagenase (destruction of connective tissue), and proteases (can prevent IgG). Leukotoxin plays an important role in pathogenicity [14].

#### METHODS

The type of this study was experimental laboratory with post-test only control group design. The production of soursop leaf extract was done at the Pharmaceutical Laboratory Faculty of Pharmacy USU. Identification of soursop leaves was conducted at Herbarium Medanense, Medan, North Sumatra. A specimen voucher has been deposited there. Bacterial sampling and testing were conducted at the microbiology laboratory, Faculty of Dental Medicine, Airlangga University, Surabaya. Based on Federer's formula, each sample is done 3 times repetition.

A total of 500 g of soursop leaf dissolved with 70% ethanol as much as 1.5 L. The process of making soursop leaf extract was done by maceration method. Examination of soursop leaf extract with Aa ATCC® 6514™ bacteria was carried out by liquid dilution method with brain heart infusion broth (BHIB) media and used two control groups,



Fig. 2: The result of gram staining of *Aggregatibacter actinomycetemcomitans* bacteria in the form of cocobacillary seen from microscope with 10×100 magnification

chlorhexidine as positive control and BHIB media as negative control. Added 5 ml BHIB media into the first tube to tube seventh and eighth tube inserted 5 ml chlorhexidine. Then, add 5 ml of soursop leaf extract into the first tube and dilution from the first tube to the sixth tube to get the concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.5625%. Then added a suspension of Aa ATCC® 6514™ of 0.1 ml into each tube whose turbidity has equalized 0.5 Mac Farland, then each of the tubes homogenized. Then, the eight tubes were inserted into the anaerobic jar and was incubated into the incubator for 24 h at a temperature of 37°C and were observed whether precipitation or not. And continued to subculture with Petri dishes containing media Muller Hinton Agar (MHA) to determine the MIC and minimum bactericidal concentration (MBC). Using a micropipette, the solution in each test tube 0.1 ml was taken and carried out spreading using a hockey stick on MHA media. All Petri dishes are inserted into the anaerobic jar and incubator for 24 h at 37°C as previously described by Basyuni *et al.* [6]. After that done the calculation of colony and performed processing data analysis using Kruskal–Wallis test and continued by least significance different (LSD) test.

#### Statistic analysis

Data were expressed as the mean±standard deviation of triplicate experimental value (n=3). The analysis was performed using Kruskal–Wallis test (p<0.05) followed by LSD test to see the significant mean difference between treatment groups. All statistical analyzes were performed using SPSS for Windows Version 23.

#### RESULTS

Dilutionally, the entire tube of each concentration was observed to be cloudy due to the extract of a dark brown. The results of the negative control obtained showed that the media turned dark and cloudy which meant that it was unable to inhibit bacterial growth so that Aa ATCC® 6514™ grow well on BHIB media. Whereas the positive controls using chlorhexidine clearly show that able to inhibit and kill bacteria. Followed by a subculture on the MHA medium to obtain a MIC and MBC score (Fig. 3).

MIC and MBC were obtained by observing the subculture results on Petri dishes containing MHA media. The growth of Aa ATCC® 6514™ bacteria was characterized by a gray colony composed of irregular and cloudy groups.

Fig. 4 is a subculture result to prove KHM and KBM concentrations using Petri dishes containing MHA media. The results obtained at concentrations of 50%, 25%, 12.5%, and 6.25% and positive controls found no colony growth, whereas at concentrations of 3.125%, 1.5625%, and negative controls obtained colony growth.

Table 1 presents that Kruskal–Wallis test showed soursop leaf extract with concentration of 50%, 25%, 12.5%, and 6.25% killing Aa ATCC®



Fig. 3: The reaction tubes with concentrations of 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.25% negative control and positive control were incubated for 24 h at 37°C

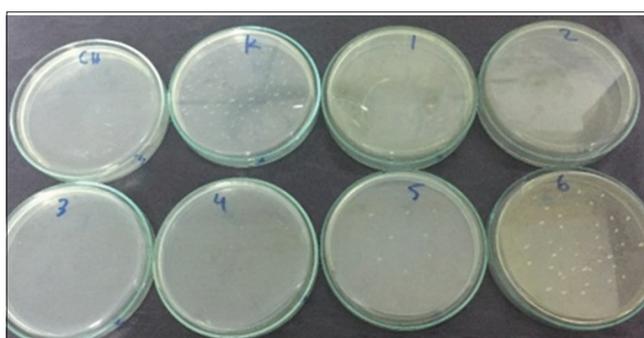


Fig. 4: First replication of positive control (CH), negative control (K), (1) concentration 50%, (2) 25%, (3) 12.5%, (4) 6.25%, (5) 3.125%, and (6) 1.5625% were incubated for 24 h at 37°C

Table 1: Number of colonies of *Aggregatibacter actinomycetemcomitans* ATCC® 6514™ bacteria with the addition of soursop leaf extract based on different concentrations

No	Concentration	N	X±SD	p value
1	50%	3	0	0.0002*
2	25%	3	0	
3	12.5%	3	0	
4	6.25%	3	0	
5	3.125%	3	10.00±1.00	
6	1.5625%	3	29.00±2.00	
7	(+)	3	0	
8	(-)	3	163.67±4.163	

Kruskal-Wallis test, \*Significant P<0.05. SD: Standard deviation

Table 2: Result of test difference of bacterial colony count of *Aggregatibacter actinomycetemcomitans* ATCC® 6514™ from each concentration of soursop leaf extract

The concentrations tested	p value against the comparison concentration							
	50%	25%	12.5%	6.25%	3.125%	1.5625%	(+)	(-)
50%	-	1	1	1	0.000*	0.000*	0.000*	1
25%	1	-	1	1	0.000*	0.000*	0.000*	1
12.5%	1	1	-	1	0.000*	0.000*	0.000*	1
6.25%	1	1	1	-	0.000*	0.000*	0.000*	1
3.125%	0.000*	0.000*	0.000*	0.000*	-	0.000*	0.000*	0.000*
1.5625%	0.000*	0.000*	0.000*	0.000*	0.000*	-	0.000*	0.000*
(+)	1	1	1	1	1	1	-	0.000*
(-)	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	-

LSD test. \*Significant (p<0.05). LSD: Least significance different

6514™ bacteria and soursop leaf extract with 1.5625% concentration inhibited the bacterial growth of Aa ATCC® 6514™, and there was a significant relationship between all concentrations of soursop leaf extracts to the amount of bacterial colonies of Aa ATCC® 6514™.

At concentration 6.25% no more visible bacterial growth Aa ATCC® 6514™, then the concentration of 6.25% shows the value of MBC soursop leaf extract. When compared to the number of bacterial colonies of Aa ATCC® 6514™ present in the negative control group (BHIB Media), the concentration of 1.5625% is MIC extract of soursop leaf extract to Aa ATCC® 6514™ bacteria. Table 1 showed that H<sub>0</sub> is accepted because soursop leaf extract has the ability to inhibit and kill bacteria Aa ATCC® 6514™. The number of bacterial colonies *Aggregatibacter actinomycetemcomitans* ATCC® 6514™ can be inhibited by the addition of soursop leaf extract with different concentrations.

In Table 2, the results of differences in the number of bacterial colonies of Aa ATCC® 6514™ from each concentration of soursop leaf extract using LSD test were significant (p<0.05). Test results showed that at concentrations of 50%, 25%, 12.5%, 6.25% and positive controls have a significant difference with concentration of 3,125% and negative controls. This can be seen concentrations of 50%, 25%, 12.5%, 6.25% and positive controls showing clear colored media, while concentration of 3.125%, 1.5625% and negative controls showing the darker media. This test further reinforces that the higher concentration of soursop leaf extracts, the less the growth of bacterial colonies. Thus, the soursop leaf extract has an antibacterial effect against the bacterium Aa ATCC® 6514™.

### DISCUSSION

The extracts of higher plant can be very good source of antibiotics against various bacterial pathogens [15]. Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against any microorganisms, insects, and other herbivores [16].

This research was conducted to find the level of effectiveness of MIC and MBC from soursop leaf extract with various concentrations of Aa ATCC® 6514™.

Aa was selected as the study sample because Aa is a Gram-negative, anaerobic facultative which is the main cause of periodontal disease, one of which is local aggressive periodontitis. The original habitat of this bacterium is the oral cavity. Other bacteria that can cause periodontal disease are *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum* which are a Gram-negative bacteria [6,17,18]. However, the most dominant bacteria found in periodontitis is the bacterium Aa [19].

Chlorhexidine was chosen as a positive control because antiseptics and disinfectants have bactericidal and bacteriostatic effects against Gram-positive and Gram-negative bacteria. BHIB was chosen as a negative control

because the BHIB medium is a liquid medium used for microorganism culture for antibacterial test, and it was expected that many colonies are formed to be a benchmark to determine MIC and MBC [20].

Table 1 shows soursop leaf extract concentration of 50%, 25%, 12.5%, and 6.25% causing no bacterial colony Aa ATCC® 6514™ which can grow (0), so the concentration of 6.25% becomes MIC extract value soursop leaves against Aa ATCC® 6514™ bacteria. When compared with the number of *Aggregatibacter actinomycetemcomitans* colonies ATCC® 6514™ in the negative control group (Media BHIB), at concentration 6.25% is less inhibit bacteria, therefore the concentration of 6.25% is MBC soursop leaf extract to Aa ATCC® 6514™. Data from Table 2 also showed that the higher concentration of soursop leaf extracts, the less the growth of Aa ATCC® 6514™ bacteria. This was caused by the greater concentration of soursop leaves extract, the greater the antibacterial content.

Table 2 shows that the differences in the number of bacterial colonies of Aa ATCC® 6514™ from each concentration of soursop leaf extract were significant. Based on the results of the research, there are bacteriostatic and bacteriocidal effects of various soursop leaf extracts on the growth of Aa ATCC® 6514™.

The antibacterial effect of soursop leaves on Aa ATCC® 6514™ was caused by the active compounds contained therein. Soursop leaf extract has a content such as alkaloids, flavonoids, tannins, steroids, and saponins that act as antibacterials [11].

The ability of alkaloid compounds as antibacterials is influenced by basic groups containing one or more nitrogen atoms. If the base group is in contact with the bacteria then, it will react with the amino acid compounds that make up the bacterial wall. This reaction leads to changes in the structure of amino acids, and bacterial DNA will be damaged. This damage will encourage bacterial lysis [21].

Flavonoids are a group of phenol compounds most abundant in nature. The biological activity of flavonoid compounds against bacteria is done by damaging the cell walls of bacteria consisting of lipids and amino acids. Therefore, the bacterial cell nucleus will also lysis [22].

Phenolic group compounds such as tannins inhibit bacterial growth by inhibiting protease enzyme activity in the bacterial cell protein transport process, as well as inactivating the function of genetic material. Tannin is also capable of shrinking bacterial cell walls, thus disrupting the permeability of cell membranes by forming tannin complexes with enzymes and bacterial substrates, thus causing the cell to not conduct live activity. This may cause the growth of bacteria to be inhibited so that the cell dies. The mechanism of action of steroids as antibacterial is by destroying bacterial cell membranes [23].

In addition, soursop leaves also contain saponin active ingredients. Saponin is a type of triterpene glycoside and sterol which is an active compound on the leaf surface. The antibacterial action mechanism of saponins by increasing the permeability of cell membranes. Saponin seems inhibit bacterial growth, which might increase the efficiency of protein synthesis of bacteria, so membrane function becomes unstable and leads to cell hemolysis [24]. However, polyisoprenoid extract from mangrove did not inhibit *Candida albicans* growth [25].

Soursop leaf extract has been shown to have an antibacterial effect on various microbes. One of them in research that has been done *in vitro* soursop leaves extract showed the effectiveness of antibacterial power to the growth of *S. mutans*, *Streptococcus mitis*, and *P. gingivalis* bacteria which can even inhibit the growth of fungus *C. albicans* [10].

This study clarified that soursop leaf extract has an *in vitro* antibacterial effect against Aa ATCC® 6514™ with minimal concentrations that inhibit bacterial growth at concentrations of 1.5625% and minimal concentrations that can kill bacteria at concentrations of 6.25%.

## CONCLUSION

Based on the results of experimental studies to determine the effect of antibacterial by finding the value of MIC and MBC, From this study we suggested the effectiveness extract of soursop leaf to Aa ATCC® 6514™ *in vitro* obtained MIC value at 1.5625% concentration and value of MBC at concentration 6.25%.

## AUTHORS' CONTRIBUTION

AP designed and performed the experiments. AP, MN, and NHA derived the models and analyzed the data. AP and DPS wrote the manuscript in consultation with MB, MN, and NHA. All of the authors read and approved the final manuscript.

## CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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