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

AMETA PRIMASARI1, MINASARI NASUTION1, NURUL HIDAYATI ARBI1, DINI PERMATA SARI2, MOHAMMAD BASYUNI3*

1Departement of Oral Biology, Fakultas Kedokteran Gigi, Universitas Sumatera Utara, Jl. Alumni No. 2 Campus USU Padang Bulan Medan 20155, Indonesia. 2Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan 20155, Indonesia. 3Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Jl. Tri Dharma Ujung No. 1 Medan, North Sumatra, 20155, Indonesia.

Email: m.basyuni@usu.ac.id

Received: 12 July 2018, Revised and Accepted: 11 August 2018

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.

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 plants 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, anti hypertensive properties, antioxidative, and anticancer effects [3-6].

Ethanol extract of soursop leaf (A. muricata L.) had antibacterial activity against ATCC® 35668™ Streplococcus 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, 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
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, annocatin, annocatilin, annomexocin, annonacin, anomurian, anomurnine, anol, cacourine, gentisic acid, gigantertron, linoleic acid, muriapentoxin, niacin, phosphorus, calcium, carbohydrate, Vitamin C, Vitamin B₂, Vitamin B₃, 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 identification of soursop leaves was conducted at Herbarium Medanense, Medan, the Pharmaceutical Laboratory Faculty of Pharmacy USU. Identification of periodontitis bacteria was 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).

**Fig. 1: Soursop leaf**

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

<table>
<thead>
<tr>
<th>No</th>
<th>Concentration</th>
<th>N</th>
<th>X±SD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50%</td>
<td>3</td>
<td>0</td>
<td>0.0002*</td>
</tr>
<tr>
<td>2</td>
<td>25%</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12.5%</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>6.25%</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3.125%</td>
<td>3</td>
<td>10.00±1.00</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.5625%</td>
<td>3</td>
<td>29.00±2.00</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>(+)</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>(-)</td>
<td>3</td>
<td>16.36±4.163</td>
<td></td>
</tr>
</tbody>
</table>

Kruskal–Wallis test. *Significant P<0.05. SD: Standard deviation

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 microbiomnism 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 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 antibacterials 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.

REFERENCES
17. Tenggara FS, Rizky Z, Parisihni K. Daya hambat ekstrak daun...


