Effectiveness of Some Concentration Star Fruit Leaf Extracts to Growth of *Streptococcus sanguis*

Minasari, Sri Amelia, Yolanda Simatupang
Oral Biology Department Faculty of Detistry
"University of North Sumatera
Padang Bulan, 2015

Abstrak

Star fruit leaves play an antibacterial that has composition of flavonoids, tannins, saponins, triterpenoids, its function destroys bacterial cells, protein denaturation, causing increased permeability of cell membranes to make bacterial cell lysis. Streptococcus sanguis serves as the attachment of other microorganisms that colonize the tooth surface, forming dental plaque, contributing to caries development, Recurrent Aftosa Stomatitis (RAS) and periodontal disease. Streptococcus sanguis serves as the attachment of other microorganisms that colonize the tooth surface, forming dental plaque, contributing to caries development, Recurrent Aftosa Stomatitis (RAS) and periodontal disease. The purpose of this research is to know the effectivity level of MIC and MBC of star fruit leaf extract on the growth of Streptococcus sanguis bacteria from concentration 60%, 40%, 20%, 10%, 5%. This research method is experimental laboratory with post-test only control group design. The effectiveness test of star fruit leaves extract on the growth of Streptococcus sanguis with dilution method. Leaves extract of star fruit made by maceration technique from each concentration then added suspension of Streptococcus sanguis bacteria, repetition done four times repetition then conducted observation. The data analysis used is one way ANOVA test. The result showed that MIC concentration of the extract on the growth of Streptococcus sanguis was 5% and MBC was 10%. The conclusion of this study, starfruit leaf extract has effectiveness against Streptococcus sanguis.

Keyword: starfruit leaf, effectiveness, MIC, MBC, *Streptococcus sanguis*,.

Introduction

Public interest shows traditional medicine is quite high (Ministry of Health Republic of Indonesia (MOH RI, 2009) as much as 34,41%. According to WHO the world's population who use traditional medicine 60%, while 40% using modern medicine from statistical data. Indonesia is the 2nd country in the world that has the diversity of plants to improve health. In this world there are 30,000 species of herbs for health, 940 species are successful as medicine, but currently only 283 species have been utilized.[1]

Starfruit (*Averrhoa bilimbi L*) is one of the plants that has not been cultivated specifically, but often used as a traditional medicine. This plant is used to overcome various diseases such as canker sores, dental pain, bleeding gums, cough, diabetes, rheumatism, mumps, acne, diarrhea, high blood pressure, and stroke. The part of the plant that I studied was the leaves, contained tannins, flavonoids, saponins, and triterpenoids as an antibacterial activity.[2]

Tannin activity as an antimicrobial through several mechanisms inhibits microbial enzymes and inhibits bacterial growth by reacting with cell membranes and inactivating essential enzymes or genetic material.[2,3]Flavonoid active compounds have the ability to form complex compounds with bacterial proteins through hydrogen bonds, causing cell wall structures and bacterial cytoplasmic membranes that contain proteins to become unstable and lose their biological activity. Furthermore, the bacterial cells will be disrupted by growth due to increased permeability of bacterial cells, and lysis and no bacterial growth occurs. Triterpenoids can damage the lipid fraction of the cytoplasmic membrane, thus disrupting the functioning of the membrane or cell wall formation.[3] Previous research Sari M (2014), young wuluh belimbing leaves 1.60% tannins and 1.8% old belimbing wuluh leaves. Research Sriherfyna FH, Zubaidah E, Chintia Devi Pendit PA (2016) leaf belimbing wuluh has antibacterial activity against Staphylococcus aureus and Escherichia coli.[5]

The active substance of the belimbing wuluh leaves as antimokroba quickly reacts with the cell membrane and inactivates the essential enzymes or genetic material.[2,5] This condition causes tension in the cell wall and cytoplasmic membrane, an increase in permeability of the cell wall so that the cell is destroyed and does not occur bacterial growth.

Picture 1. Star fruit leaf
(Documentation)

Classification of Star fruit

Starfruit (*Averrhoa bilimbi* L) belongs to the species of the *Averrhoa* family.[2,4] Taxonomically starfruit can be classified as follows:[5]

- Kingdom : *Plantae* (plants)
- Subkingdom : *Tracheobionta* (vicious)
- Superdivisio : *Spermatophyta* (produce seeds)
- Divisio : *Magnoliophyta* (flowering)
- Class : *Magnoliopsida* (dicoted)
- Sub-class : *Rosidae*
- Ordo : *Geraniales*
- Familia : *Oxalidaceae* (starfruit)
- Genus : *Averrhoa*
- Spesies : *Averrhoa bilimbi L*

Star fruit morfology

Carambola star fruit can live at an altitude of 5-500 meters above sea level, the height reaches 5-10 meters with a short main stem. Branching a little, the location of low branches, bumpy and stem diameter about 30 cm direction leaning upward, young branches with smooth hair like velvety brown. Flowers in the form of jasmine, in groups, out of a large trunk or branch. Flower small star-shaped, reddish purple color. Star fruit shaped ellipse like torpedo, length 4-10 cm.[2,4]

Young fruit green with the rest of the petals stuck to the end. While the ripe fruit is yellow or pale yellow, the flesh is watery and very sour. Sour taste is often used as a spice cooking and mixture of herbs, shiny and thin fruit skin. Seeds are oval-shaped measuring 6 mm, shaped flat, and brown, and covered with mucus. This tree grows in places exposed to direct sunlight and quite moist. Almost all parts of the belimbing wuluh can be utilized, one of them is the leaf part.[2,4]

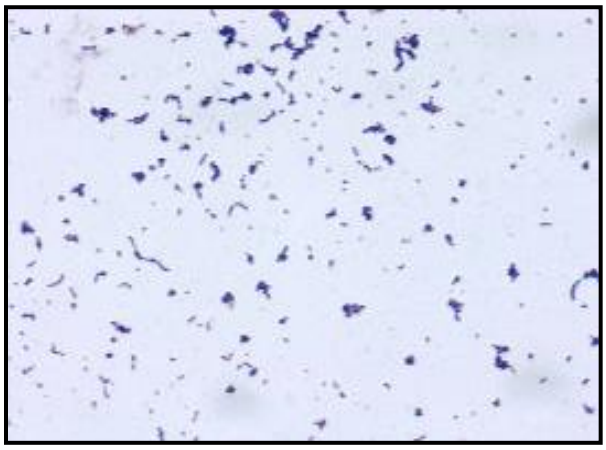
Leaves of wuluh wuluh compound, odd pinnate with 21 to 45 pairs of split or half-pairs and oval-shaped leaves. Short-stemmed leaves, rounded oval, pointed, rounded base, flat edge, 2-10 cm long, 1-3 cm wide, the meat is paper-thin and thin, The upper surface is dark green and there are fine hairs and the lower surface of the color is younger.[4]

Another study proved extract of starfruit leaves with concentrations of 2.5%, 5%, 10%, 20%, 40% had an effect on the inhibition zone of *Streptococcus sanguis* growth.

This research uses diffusion method that is by the technique of wells that have been spiced leaf extract of star fruit. The clear zone formed on the edge of the well proves the antibacterial power generated by star fruit leaf extract (*Averrhoa bilimbi* L) in inhibiting the growth of *Streptococcus sanguis* bacteria.[6]

Another study on the effectiveness of antibacterial powder of starfruit leaf (*Averrhoa bilimbi* L) on the growth of *Streptococcus mutans* bacteria. An antibacterial activity test was performed to determine the extent of the inhibitory zone around the well. The results obtained are star fruit leaf poppy (*Averrhoa bilimbi Linn*) can inhibit the growth of *Streptococcus mutans* bacteria. This can be seen from the drag zone in the area around the wellbore and the more heavy the leaf of star fruit (*Averrhoa bilimbi Linn*) the stronger the drag.[2]

Streptococcus sanguis



Picture 2. Gram staining shows characteristicsof *Streptococcus sanguis* [7]

***Streptococcus sanguis* classification**

*Streptococcus sanguis*based on the wall structure is a gram-positive bacteria, diameter 0,5-1µm. *Streptococcus sanguis* is a gram-positive bacterium in the form of coccus (round) with diameter 0,6 – 1,0 µm arranged like a chain. *Streptococcus sanguis* is non motile, negative catalase, grows optimum at 37 ° C with pH 7.4-7.6, opa colored, the surface is rough (only 7% is mucoid). *Streptococcus sanguis* is a type of alpha-type *Streptococcus hemoliticus* bacteria called a nonhemolytic strain that can normally be found in the oral cavity. The observations under the microscope are round and arranged like chains. Based on the science of taxonomy, *Streptococcus sanguis* bacteria are classified as follows:[8]

- Kingdom : *Bacteria*
- Filum : *Firmicutes*
- Class : *Bacilli*
- Ordo : *Lactobacilalles*
- Famili : *Streptococcaceae*
- Genus : *Streptococcus*
- Spesies : *Streptococcus sanguis*

Based on the need for oxygen, *Streptococcus sanguis* is classified on facultative anaerobic bacteria when contact with oxygen can still continue its growth. However, when oxygen is less than required when metabolism, the bacteria can ferment with the aid of synthesis of Adenosine triphosphate (ATP).[9,10] These bacteria colonize on the tooth surface and mucous membranes of the oral cavity, thus forming biofilm.[9]

Materials and Methods

This type of research is experimental laboratory with post-test only control group design. That is a measurement or observation after treatment is given. Place and time of research star fruit leaf extract is done at the Laboratory of Traditional Medicines of Faculty of Pharmacy USU, identification, breeding and testing of samples from June to July 2017 conducted in Hospital Clinic Microbiology Unit of USU. The research sample that will be used is Streptococcus sanguis that is identified and cultured in the Unit of Clinical Microbiology of USU Hospital. large sample of experimental research used Federer's formula. Federer's large sample formulas are:

$$(t-1)\,(r-1) \geq 15$$

Where t = amount of treatment and r = number of replication
This research uses 7 treatment groups, they are:
1. Group 1 : star fruit leaf extraxt 60 %
2. Group 2 : star fruit leaf extraxt 40 %
3. Group 3 : star fruit leaf extraxt 20 %
4. Group 4 : star fruit leaf extraxt 10 %
5. Group 5 : star fruit leaf extraxt 5 %
6. Group 6 : Chlorhexidine as a positive control
7. Group 7 : Aquabides as a negative control

Thus, the treatment amount (t) = 7, then

$$(t - 1)\,(r - 1) \geq 15$$
$$(7 - 1)(r - 1) \geq 15$$
$$r - 1 \geq 2{,}5$$
$$r \geq 3{,}5$$
$$r \geq 4$$

The required number of samples is 1 sample with replication 4 times, meaning that in groups 1-7 done each 4 repetitions to prevent the occurrence of bias.

Star fruit leaf extraxt Manufacture

Preparation of Starfruit Leaf

1. Star fruit leaf 1.5 kg, then cleaned by washing under running water until clean and then dried in a way diangin-aired.
2. Dried if star fruit leaf are pollinated using a blender.
3. Then stored in a sealed container.

Picture 3. Drying of star fruit leaves (documentation)

Extraction Process of Powder Starfruit Leaf

1. Mix the leaves of starfruit leaves with ethanol, stirring for ± 15 minutes for 5 days.
2. Attach the maseration bottle and connect with the faucet appropriately. Then put the cotton into the end of the bottle and solidify. Above cotton is placed a round filter paper so that it coats the bottom of the bottle.
3. Then mixing the powder and ethanol into the filter tube, holding the liquid extract in one container.

4. Turn on the water machine and turn the faucet so that water will go into the rotapavor tool. Insert the result of maceration into the sample flask and put the flask in place. Turn on the heater, adjust the temperature by pressing the set button and adjust the temperature by pressing the up and down button.
5. Open the handle position to unlock and lower the pumpkin until submerged liquid is approximately ½ of the size of the pumpkin. Return the handle position to the lock position.
6. Turn on the vacuum and cover the vacuum faucet. Refill the maseration when it is reduced.
7. Move the container flask if it is full of ethanol. Do not forget to open the vacuum taps before opening any pumpkins.
8. After the maceration becomes thick as melted chocolate, stop the rotavaporation process and transfer it to a container.
9. Dilute the thick extract with ethanol to obtain the extract of starfruit leaves with concentrations of 60%, 40%, 20%, 10%, and 5%.

Results

Effectiveness of star fruit leaf extraxt to growth of *Streptococcus sanguis*

MIC concentration was obtained from observing the cloudy tube subculture results in the TYC petri dish (Tryptone Yeast Cysteine). TYC petri dishes with the lowest concentrations can inhibit the growth of bacterial colonies showing MIC concentrations. The growth of *Streptococcus sanguis* bacteria is characterized by the presence of spherical colonies, shiny, smooth, arranged in irregular, white or cloudy groups.

Table 1. The test results of MIC and MBC concentration of leaf belimbing wuluh extract

Streptococcus sanguis on TYC media

1	Star fruit leaf extract 60%	-	-	-	-
2	Star fruit leaf extract 40%	-	-	-	-
3	Star fruit leaf extract 20%	-	-	-	-
4	Star fruit leaf extract 10%	-	-	-	-
5	Star fruit leaf extract 5%	+	+	+	+
6	Chlorhexidine 0,1%	-	-	-	-
7	Akuabides	+	+	+	+

Information : (+) = there is colony growth
(-) = there is not colony growth

Table 1 shows that a 5% MIC concentration in which there is still a growth of bacterial colonies. MBC concentration is obtained from observing the cloudy tube subculture results in TYC petri dish. TYC petri dishes with the lowest concentrations of bacterial colony growth showed no concentrations of MBC. Concentrations of 60%, 40%, 20%, and 10% have no bacterial colony growth. The lowest concentration was found in the concentration of 10% of wuluh leaf belimbing extract which showed the concentration of MBC.

Table 2. Different test results Amount of bacterial colonies from some concentration of leaf starfruit extract against *Streptococcus sanguis* on TYC media

Petri	Test Material	Repetition 1	Repetition 2	Repetition 3	Repetition 4	$\bar{X}$±SD	P
1	Star fruit leaf extract 60%	0	0	0	0	0±0,000	0,000*
2	Star fruit leaf extract 40%	0	0	0	0	0±0,000	
3	Star fruit leaf extract 20%	0	0	0	0	0±0,000	
4	Star fruit leaf extract 10%	0	0	0	0	0±0,000	
5	Star fruit leaf extract 5%	21	17	24	22	21±2,944	
6	Chlorhexidine 0,1%	0	0	0	0	0±0,000	
7	Akuabides	325	354	385	356	355±24,509	

One way ANOVA test, *signifikan p<0,05

The number of colonies with the first repetition of 21, the second repetition of 17, the third repetition of 24, the fourth repetition of 22 (Table 2). The number of bacterial colonies at four repetitions was 0 (Table 2). One way ANOVA test was used to test the difference of bacterial colonies in some concentrations of wuluh belimbing leaf extract, chlorhexidine and Iabides. From the test results it is found that there are significant differences in each concentration. The significant difference is in the concentration of 5% with p = 0.000 (Table 2). Data at a concentration of 5% were found to be normally distributed using the Shapiro Wilk test.

Table 3. Different test results The amount of bacterial colony *Streptococcus sanguis* from one concentration to some concentration of star fruit leaf extract on TYC media

Tested Consetration	The value of p against the comparison concentration						
	60%	40%	20%	10%	5%	Chlorhexidine	akuabides
Star fruit leaf extract 60%	-	1	1	1	0,004*	1	0,000*
Star fruit leaf extract 40%	1	-	1	1	0,004*	1	0,000*
Star fruit leaf extract 20%	1	1	-	1	0,004*	1	0,000*
Star fruit leaf extract 10%	1	1	1	-	0,004*	1	0,000*
Star fruit leaf extract 5%	0,004*	0,004*	0,004*	0,004*	-	0,004*	0,004*
Chlorhexidine 0,1%	1	1	1	1	0,004*	-	0,000*
Akuabides	0,000*	0,000*	0,000*	0,000*	0,000*	0,000*	-

Post Hoc test , *signifikan p<0,05

After one-way ANOVA test, *Post Hoc* test was done as a follow-up test to see the difference of bacterial colony from one concentration to other concentration. The results of the *Post Hoc* test showed that the concentrations of 60%, 40%, 20%, 10%, and chlorhexidine (positive controls) had a significant difference with 5% concentration and Iabides (negative control). With each value p = 0.004 and p = 0,000 (Table 3).

Conclusion

From the results of the study "The Effectiveness of Concentration Star fruit Leaf Extract on *Streptococcus Sanguis* Growth, MIC is 5% and MBC is 10% true leaf star fruit has effectiveness on the growth of *Streptococcus Sanguis*.

References

Badan Penelitian dan Pengembangan Kesehatan Kementerian Kesehatan RI. Riset Kesehatan Dasar (RISKESDAS) 2013. Jakarta: 2013:118-9

Liantari DS. Effect of wuluh starfruit leaf extract for *Streptococcus mutans* growth. J Majority 2014; 3(7): 27-33.

Sriherfyna FH, Zubaidah. Karakteristik fisik-kimia dan aktivitas antibakteri ekstrak daun belimbing wuluh *(Averrhoa bilimbi L.)*. Jurnal pangan dan agroindustri 2016; 4(1): 400-9.

Sari M, Suryani C. Pengaruh ekstrak daun belimbing wuluh (*Averrhoa Bilimbil* L) dalam menghambat pertumbuhan jamur candida albicans secara in vitro. In: Jurusan Biologi FMIPA Universitas Negeri Medan. Prosiding Seminar Nasional Biologi dan Pembelajarannya, 2014: 325-32.

Yulianingtyas A, Kusmartono B. Optimasi volume pelarut dan waktu maserasi pengambilan flavonoid daun belimbing wuluh (*Averrhoa Bilimbi* L). Jurnal Teknik Kimia 2016; 10(2): 58-64.

Zheng W, Tan TK, Paterson IC, Siow CC, Tan SY, Old LA. Streptobase: an oral *Streptococcus mitis* group genomic resource and analysis platform. PloS ONE 2016; 11(5):1-19.

Suwandi T. Pengembangan potensi antibakteri kelopak bunga *Hibiscus sabdariffa* L.(Rosela) terhadap *Streptococcus sanguinis* penginduksi gingivitis menuju obat herbal terstandar. Disertasi: Jakarta: Universitas Indonesia, 2012:19-22.

Ge X, Kitten T, Chen Z, Lee SP, Munro CL, Xu P. Identification of *Streptococcus sanguinis* genes required for biofilm formation and examination of their role in endocarditis virulence. Infecttion And Immunity 2008;76(6):2551–9.

Anggraini N, Saputra O. Khasiat belimbing wuluh *(Averrhoa bilimbi L.)* terhadap penyembuhan *Acne Vulgaris*. J Majority 2016; 5(1): 76-80.

Brooks GF, Butel JS, Ornston LN, Jawetz E, Melnick JL, Adelberg EA. Mikrobiologi kedokteran. Alih bahasa. Edi N, RF Maulany Jakarta: Penerbit Buku Kedokteran, 1996: 219.