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ANTIBACTERIAL ACTIVITY OF MIXED MAULI BANANA STEM AND BASIL LEAVES AGAINST *Staphylococcus aureus*

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ABSTRACT
Background: Mauli banana is a typical plant in South Kalimantan which contains tannins (67.59%), saponins (14.49%), alkaloids (0.34%), and flavonoids (0.25%) as natural antiseptics inhibiting the growth of *Streptococcus mutans*. Basil is plant that contain eugenol (70%), flavonoids (4.75%), tannins (4.6%), and alkaloids (1%) as natural antiseptics inhibiting the growth of *Candida albicans.* This study was to examine the antibacterial activity of a mixture of banana mauli stem stems and basil leaves against *Staphylococcus aureus*. **Purpose:** to analyze the antibacterial activity against *Staphylococcus aureus* in the mixture of banana mauli stems and basil leaves extracts. **Method:** true experiment using pretest and posttest with control group design with 11 treatments consisting of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, and aquadest. Antibacterial activity test was conducted using dilution method. Determination of Minimum Inhibitory Concentration (MIC) was determined by contrasting the absorbance value before and after incubation. Minimum Bactericidal Concentration (MBC) was obtained by testing the concentration with negative absorbance value on MSA media incubated for 24 hours. **Results:** The results of this study indicated that the concentration of 40%, 50%, 60%, 70%, 80%, 90%, and 100% could inhibit the growth of *Staphylococcus aureus* but these concentrations had yet been able to kill *Staphylococcus aureus.* **Conclusion:** The MIC of mauli banana and basil leaves extract combination against S aureus presentts at 40% concentration yet MBC cannot be obtained.

Keywords **:** Antibacterial activity, Basil leaf, Dilution method, Mauli banana stem, *Staphylococcus aureus*
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INTRODUCTION

Staphylococcus aureus is a gram-positive bacteria and normal flora in the oral cavity.[24] These bacteria will become opportunistic pathogens if there is an imbalance of temperature, humidity, and nutrition that can lead to the formation of more colonies in the oral cavity.[4,24] Pathological conditions in the oral cavity can be caused by opportunistic pathogens, and may be manifested as angular cheilitis, facial cellulitis, osteomyelitis of the jaw, parotitis, stomatitis, hyperdontia, acute necrotizing ulcerative gingivitis, etc.[4,9] Those infections will be difficult to manage because *Staphylococus aureus* as the causative agent show resistant properties against some conventional antiseptics.

During this time many conventional antiseptics are circulated within the community. One that is widely used is *Povidone iodine* which that aims to reduce the number of pathogenic bacterial colonies in the oral cavity. Some side effects may arise in the use of *Povidone iodine*, namely sensitivity, local erythema, pain, erosion of the mucosa, and the risk of thyroid function.[17]

Mauli banana (*Musa acuminate*) is a typical plant from South Kalimantan that can be applied as a natural antiseptic.[19] The main ingredients contained in the banana mauli stem are tannins (67.69%), saponins, (14.49%), alkaloids (0 , 34%), and flavonoids (0.25%).[3] Previous studies conducted by Apriasari et al (2013) have proven that extracts of banana mauli stem at a concentration of 25% can inhibit the growth of *Streptococcus mutans*.[2] The results of Apriasari's research (2014) show that mauli banana extract at a concentration of 80% has the ability to inhibit the growth of *Streptococcus mutans*.[1] Research by Noor and Apriasari (2014) also proved that mauli banana extract at a concentration of 100% is capable to inhibit *Streptococcus mutans.*[13]

Basil leaves are plants that have potential as natural antiseptics that contain eugenol (70%), flavonoids (4.75%), tannins (4.6%), alkaloids

(1%).[7,8,14,16] Previous research conducted by Mittal et al. (2018) showed that basil leaf extract has an antibacterial activity of *Staphylococcus aureus* and *Klebsiella pneumonia* at a concentration of 100% and 25% can kill *Candida albicans.*[14]

According to Machado (2012), tannin content in plants can provide the ability to inhibit bacterial growth, while eugenol content in plants can provide the ability to inhibit fungal growth. This means that their combination can generate antibacterial property.[10] This study aims to analyze the antibacterial activity against *Staphylococcus aureus* after the administration of stem Mauli banana stem and basil leaves extracts mixture at the concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, and distilled water using the dilution method.

MATERIAL AND METHODS

This research was conducted after obtaining the research permit and ethical eligibility No. 094 / KPEPKG-FKGULM / EC / I / 2020 that was issued by the ULM Faculty of Dentistry Ethical Commission. This study used a True Experimental design (pure laboratory) with a pretest and a post-test using control group design and random sampling technique consisting of 11 treatment groups. Treatment I was given a mixture of mauli banana stem and basil leaves at a concentration of 10%, treatment II was given a mixture of mauli banana stem and basil leaves at a concentration of 20%, treatment III, was given a mixture of banana mauli stem and basil leaf extract at a concentration of 30%, treatment IV was given an extract the mixture of Mauli banana stems and basil leaves at a concentration of 40%, treatment V was given a mixture of extracts of Mauli banana stems and basil leaves at a concentration of 50%, treatment VI was given a mixture of banana Mauli stems and basil leaves at a concentration of 60%, treatment VII was given a mixture of banana mauli stems and extracts basil leaves at a concentration of 70%, treatment VIII given a mixture of banana Mauli stem extract and basil leaves at a concentration of 80%, IX treatment was given a mixture of banana mauli stem extract and basil leaves at a concentration of 90%, treatment X was given a mixed extract of banana mauli stem and basil leaves at a concentration of 100% , XI treatment was given aquadest or distilled water. Each treatment is repeated 3 times based on the calculation of Federer formula. The population used in this study is the pure isolate of Staphylococcus aureus ATCC® 6538 ™ obtained from Airlangga University Research Center. This research was conducted at the Biomedical Laboratory of the Faculty of Dentistry, Lambung Mangkurat University.

The Making of Mauli Banana Stem and Basil Leaves Extract

Mauli banana stems were obtained from the SMK PP Banjarbaru. Mauli banana stems with a pure weight of 12 kg were taken from a 1 year old tree that has produced fruits. Mauli banana stems are washed, cut into small pieces and then dried using an oven at 40 - 50° C for 5 days. Dried Mauli banana stems was mashed using a blender to form a simplicia powder weighing 600 g. Simplicia powder of Mauli banana stems was soaked with 70% ethanol in a closed container for 3 x 24 hours. Stirred and filtered occasionally, the filtrate was then kept for 4 days to precipitate the dissolved substances. The extraction results were evaporated with a rotary evaporator at 40ºC and were re-evaporated using a waterbath until a thick extract of the Mauli banana stem was collected in a total of 48.54 g.

Basil leaves were obtained from the Basil Garden located on Jl. Kurnia, Banjarbaru, South Kalimantan. Basil leaves were picked with the criteria of 2 months old, blooming, forming yellow seeds as much as 2 kg. Basil leaves are washed and dried indoors for 2 x 24 hours. The dried basil leaves were then blended into a 210 g of simplicia powder and soaked in 70% ethanol for 3 x 24 hours. The filtering process included the usage of filter paper and kept for 3 days to precipitate the dissolved substances. The extraction results were evaporated twice, first using a rotary evaporator at 40º C then using a water bath to obtain 13.44 g basil leaf thick extract with a concentration of 100%.

The results of mauli banana stem extract and basil leaves were proceeded for free ethanol testing with the addition of potassium dichromate ($K^2Cr^2O^7$) solution. Banana mauli stem extract at a concentration of 25% and basil leaf extract at a concentration of 12.5% were each obtained from a mixture of thick extracts of mauli banana stem and basil leaf extract of 100% concentration.

The Mixing of Mauli Banana Stem Extract and Basil Leaves

Concentrated solution from a mixture of banana mauli stem extract and basil leaf extract with 100% concentration was obtained from 25% banana mauli stem extract and 12.5% basil leaf extract which had been mixed with a volume ratio of 1:1. A mixture of banana mauli stem extract mixture and 100% basil leaves extract was diluted with sterile aquadest to a concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% based on the following formula:

$$V1 \times C1 = V2 \times C2$$

Explanation:
V1 = initial volume
C1 = initial concentration
V2 = final volume
C2 = final concentration

Antibacterial Test for *Staphylococcus aureus* using the Dilution Method

Staphylococcus aureus bacteria was inoculated into BHI-B media up to *McFarland* turbidity standard at 0.5 density. As much as 1 ml bacteria inoculation was put into 11 test tubes which contained a mixture of Mauli banana stem and basil leaves extract at a concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% and 1 ml of sterile distilled water.[24]

The minimum inhibitory concentration (MIC) is determined by the difference in the absorbance value (OD) after incubation subtracted with the absorbance value before incubation from various groups of concentration. The determination of the absorbance value was using UV-Vis BKD 560 spectrophotometer. The lowest concentration that inhibits bacterial growth is indicated by negative ΔOD because the OD value after incubation <before incubation.[20]

The Minimum Bactericidal Concentration (MBC) was determined by taking 0.1 ml of the concentration with negative ΔOD and then added to a petri dish containing sterile MSA media. Incubation for 24 hours at 37^{O}C was held in an aerobic incubator. Bacterial colonies number was calculated using a colony counter. The method for bacterial calculation can be done by using markers which when touched on a petri colony counter will bring up the number of colonized bacteria. If the calculation result of bacterial colonies number is 0 CFU/ml, then the MBC is obtained.[11]

RESULT

The results of the minimum inhibitory concentration test extract of banana mauli stem stems and basil leaves against *Staphylococcus aureus* can be seen in table 1.

Table 1 shows that the difference in absorbance values at the concentration of 10%, 20%, 30%, and aquadest is positive. This means that these concentrations cannot inhibit the growth of *Staphylococcus aureus* since the colonies growth still persists. Meanwhile, the difference in absorbance values at the concentration of 40%, 50% , 60%, 70%, 80%, 90%, and 100% is negative which means that these concentrations can inhibit the growth of *Staphylococcus aureus.*

MIC data gained from antibacterial activity test of mauli banana stems and basil leaves mixed extract against *Staphylococcus aureus* were tabulated and the normality test using *Saphiro-wilk* obtained a significance value of p>0.05 which means the data were normally distributed. The data was then carried out with Levene's test to identify its homogeneity which obtain a significance value of 0.003 (p <0.05). This revealed that the data were not homogeneous. One Way ANOVA test resulted in p value = 0.000 (p <0.05) showing that there were several groups that had significant differences.

Table 1. The results of the Minimum Inhibitory Concentration of mauli banana stems and basil leaves extract mixture against *Staphylococcus aureus*

Groups	N	After Incubation	Before Incubation	Difference
10%	3	0.22800	0.12633	0.10167
20%	3	0.37100	0.23900	0.13200
30%	3	0.43400	0.34533	0.08867
40%	3	0.42533	0.56433	-0.13900
50%	3	0.54400	0.69267	-0.14867
60%	3	0.61267	0.82933	-0.21667
70%	3	0.71333	0.96033	-0.24700
80%	3	0.92400	1.16467	-0.24067
90%	3	1.08467	1.33267	-0.24800
100%	3	1.23733	1.47100	-0.23367
Negative control	3	1.56600	0.47450	1.08000

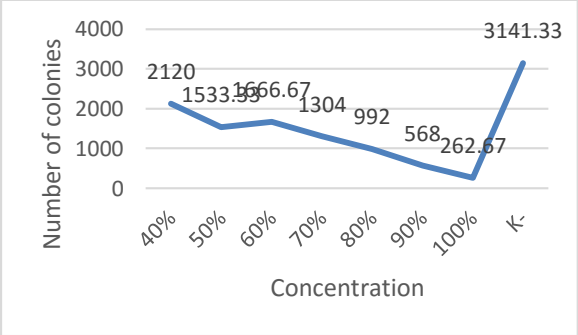

Figure 1. The average number of *Staphylococcus aureus* colonies from each treatment.

Figure 1 shows that the number of *Staphylococcus aureus* colonies in each treatment group tended to decrease if the concentration was rised except at a concentration of 60% which increased the number of colonies when compared to the number of colonies at a concentration of 50%.

Data for the average value of *Staphylococcus aureus* colonies number from each treatment was tabulated and normal test was performed using *Saphiro-Wilk* test. The significance value of p> 0.05 was obtained, which means the data were normally distributed. Then a homogeneity test was performed using *Levene's test*, that obtained a significance value of 0.001 (p <0.05) which means that the data variance was not homogeneous. Furthermore, the data were analyzed using One Way ANOVA test, obtained a significance value of 0,000 (p <0.05) which indicates a significant difference between the treatment groups.

DISCUSSION

Based on the results, it can be seen that the extract of a mixture of Mauli banana stems and basil leaves at a concentration of 10%, 20%, 30% cannot inhibit the growth of *Staphylococcus aureus.* It may occur because of the large difference of inhibition for each bacteria test concentration, where the higher the concentration, the more the inhibition will also increase. That is caused by the increase in active compounds contained in the extract or test liquid.[18]

A mixture of mauli banana stem and basil leaves at a concentration of 40%, 50%, 60%, 70%, 80%, 90%, and 100% has been found to inhibit the growth of *Staphylococcus aureus*, marked by a decrease in absorbance value, so that at a concentration of 40 % is determined as the minimum inhibitory concentration (MIC) of the growth of *Staphylococcus aureus*. This is related to the content of tannin and eugenol as the largest secondary metabolites of the mauli banana stem and basil leaves. Tannins are complex compounds of phenols, polyphenols, and flavonols which have antimicrobial activity.[2] Tannins can destroy polypeptides in cell walls and also work through the denaturation process of protein. Protein denaturation occurs due to the formation of H^+ ions from tannin bonds with microbial proteins so that the enzymes are activated in microbial cells and the process of microbial metabolism will be disrupted.[1]

Eugenol is a compound that is mostly contained in basil leaves. It will work by damaging the cell wall in *Staphylococcus aureus* so that lysis occurs in the bacterial cell wall. This will cause the leak of protein, lipids, potassium, and ATP from the cell wall so that the bacterial growth will be inhibited.[6] A different mechanism was proposed by Das et al (2019) where eugenol is able to trigger cell cytotoxicity because it can produce reactive oxygen species (ROS) that can induces inhibition of cell growth, disruption of cell membranes, and DNA damage resulting in bacterial cell decomposition.[4]

At the concentration of 40%, 50%, 60%, 70%, 80%, 90%, and 100%, the mixture was proven to inhibit *Staphylococcus aureus* growth, but no bactericidal activity was yet observed. According to Pelezar and Chan, there are several factors that can influence how an antibacterial substance works, namely: a) the concentration of antibacterial substances contained, where the higher the concentration of an antibacterial agent, the higher the antibacterial power, which means that more antibacterial substances will kill more bacteria faster; b) the number of microorganisms, it takes longer time to kill microorganisms if the number of microorganisms increase; c) microorganism species, each species of microorganism shows different resistance to a certain antibacterial agent.[22] The minimum inhibitory concentration is influenced by the structure and composition of the tested bacterial cell.[5] *Staphylococcus aureus* is a gram-positive bacterium containing polysaccharides and antigenic proteins, and also an important substance in the structure of cell walls. The cell wall of *Staphylococcus aureus* is also composed of a relatively thick layer of peptidoglycan that is surrounded by the celloic acid.[23] Peptidoglycan and polysaccharide polymers form a rigid cell wall, in which the teichoic acid formulate a link between peptidoglycan and antigens.[5] That composition of the cell wall causes inhibition of antibacterial substances to enter the cell wall of *Staphylococcus aureus*.[23]

In this study, the mixture of Mauli banana stems and basil leaves at a concentration of 100% represents the best concentration that has the best antibacterial efficacy when observed from the absorbance value. According to Putri and Milanda (2016), the type and the level of concentration given can affect the performance of antibacterial substances. The level of inhibition of an antibacterial agent is always directly proportional to the amount of concentration given.[15]

The results of the research that have been conducted show that the extract of mauli banana when combined with basil leaves extract will experience a decrease in antibacterial activity against *Staphylococcus aureus* when compared to the use of a single extract. Previous studies have shown that mauli banana extract at a concentration of 25% can inhibit the growth of *Streptococcus mutans* and basil leaves at a concentration of 12.5% can inhibit the growth of *Candida albicans*.[2,14]

The theory was revealed by Sukandar who claimed this extract combination as synergistic. This happens when the extract combination experiences an increase in antibacterial activity with a smaller concentration than when it is used singly. An extract combination is called antagonist when the extract combination used have decreased the antibacterial activity.[21]

From the results of this study it can be concluded that the MIC from a mixture of banana mauli stems and basil leaves extracts on the growth of Staphylococcus aureus has the highest killing activityat at a concentration of 40%, but MBC in this study has not been found.

REFERENCES

1. Apriasari ML. Potensi Batang Pisang Mauli (Musa acuminata) sebagai Obat Topikal pada Penyembuhan Luka Mulut. Banjarbaru: *Grafika Wangi Kalimantan*; 2014. p. 43-51.
2. Apriasari ML, Fadhilah A, Carabelly NA. Aktivitas antibakteri ekstrak etanol batang pisang mauli (Musa sp) terhadap *Streptococcus Mutans. Dentino*. 2013; 12 (3): 150.
3. Carabelly AN, Aspriyanto D, Apriasari ML, Rizky S. The toxicity of mauli banana *(Musa acuminata)* stem water extract on bone marrow mesenchymal stem cell in vitro. *International Journal of Applied Pharamaceutics.* 2019; 11 (1): 184.

4. Das M, Sabuj AAM, Haque ZF, Barua N, Pondit A, Mahmud MM, et al. Characterization of *Staphylococcus aureus* isolated from human dental infection. *African J Microbiology Research*. 2019. 13 (14): 273-274.
5. Dewi AK. Isolasi, Identifikasi dan Uji Sensitivitas Staphylococcus aureus terhadap Amoxicillin dari Sampel Susu Kambing Peranakan Ettawa (PE) Penderita. *Jurnal Sain Veteriner*. 2013; 31 (2): 142.
6. El-baky RMA, Hashem ZS. Eugenol and Linalool: comparison of their antibacterial and antifungal activity. *African Journal of Microbiology Research*. 2016: 10 (44): 32.
7. Garg P, Garg R. Phytochemical screening and quantitative estimation of total flavonoids of *Ocimum sanctum* in different solvent extract. *The Pharma Innovation.* 2019; 8(2): 16-21.
8. Gupta BM, Gupta R, Agarwal A, Goel S. *Ocimum sanctum* (medical plant) research: a scientometric assessment of global publications output during 2008-17. *International Journal of Information Dissemination and Technology*. 2018; 8 (2): 68.
9. Loon YK, Satari MH, Dewi W. Antibacterial effect of pineaple (*Ananas comous*) extract towards *Staphylococcus aureus*. *Padjajaran J Dentistry*. 2018; 30 (1): 1.
10. Machado JA, Rebelo MA, Favaro LIL, Vila MMDC, Gerenutti M. In vitro evaluation of the antimicrobial potential association of *Schinus Terebinthifolius Raddi* and *Syzygium aromaticum L*. *IOSR Journal of Pharmacy*. 2012; 2(3): 442.
11. Mama M, Teshome T, Detamo J. Antibacterial activity of honey against *Methicillin-Resistant Staphylococcus aureus:* A laboratory-Based experimental study. *International Journal of Microbiology*. 2019; 2019: 3.
12. Mittal R, Kumar R, Chahal HS. Antimicrobial activity of *Ocimum sanctum* leaves extract and oil. *Journal of Drug Delivery and Therapeutics*. 2018; 8 (6): 204
13. Noor MA, Apriasari ML. Aktivitas antibakteri ekstrak etanol batang pisang mauli (*Musa acuminata*) dan *povidone iodine* 10% terhadap *Streptococcus mutans*. *Jurnal PDGI*. 2014; 63 (3): 78-79.
14. Ornay AKD, Prehananto H, Dewi ASS. Daya hambat pertumbuhan *Candida albicans* dan daya bunuh *Candida albicans* ekstrak daun kemangi (*Ocimum sanctum I.). Jurnal Wiyata*. 2017; 4 (1): 82.
15. Puteri T, Milanda T. Uji Daya Hambat Ekstrak Daun Lidah Buaya (*Aloe vera L.)* Terhadap Bakteri *Escherichia coli* dan *Staphylococcus aureus*: Review. *Farmaka*. 2016; 14(2): 9-17.
16. Raghav PK, Saini M. Antimicrobial properties of tulsi (*Ocimum sanctum*) in relation to shelf life enhancement of fruits & vegetables. *International Journal of Green and Herbal Chemistry*. 2018; 7 (1): 21.
17. Rifdayani N, Budiarti LY, Carabelly AN. Perbandingan efek bakterisidal ekstrak mengkudu (*Moria Citrifolia Liin*) 100% dan *Povidone Iodine* 1% terhadap *Streptococcus mutans in vitro*. *Dentino*. 2014; 2 (1): 2.
18. Rusli, Hafid M, Badjadji NN. Uji Efektivitas Antibakteri Kombinasi Ekstrak Daun Paliasa (*Kleinhovia Hospita L*) Varietas Bunga Putih Dan Bunga Ungu Terhadap Pertumbuhan *Staphylococcus aureus* dan *Escherichia coli*. *Media Farmasi*. 2018; 14 (1): 8-13
19. Saputri MV, Carabelly AN, Firdaus IWAK. Toxicity test of the mixed mouthwash ash of mauli banana stem and basil leaf againts fibroblast cell study *in vitro*. *Dentino*. 2019; 4 (2): 151-152.
20. Septama AW, Panichayupakaranant P. Antibacterial activity of artocarpanone isolated from Artocarpus heterophyllus heartwoods against diarrheal pathogens and its mechanism of action on membrane permeability. *Journal of Applied Pharmaceutical Science.* 2017; 7 (11): 065.
21. Sukandar EY, Garman AN, Khairina C. Uji Aktivitas Antimikroba Kombinasi Ekstrak Perikarp Manggis (*Garcinia mangostana L.)* dan Kelopak Bunga Rosella (*Hibiscus sabdariffa L.)* terhadap Bakteri Penginfeksi Kulit. *Acta Pharmaceutica Indonesia.* 2014; 39(3): 57-62.
22. Sulistijowati R, Mile L, Wulandari K. Aktivitas Antibakteri Kitosan Kulit Udang Vaname (*Litopenaeus vannamei*) Terhadap Bakteri Kontaminan Bakso Ikan Tuna (*Thunnus Sp.). Universitas Negeri Gorontalo*. 2015; 00(00): 1-7.
23. Sumiati E. Uji Aktivitas Antibakteri Ekstrak Kloroform dan Ekstrak Etanol Biji Bidara Laut (*Strychnos ligustrina Bl*) *Terhadap Staphylococcus aureus* ATCC 25923 dan *Salmonella thypi*. *Jurnal Ilmiah Biologi.* 2014; 2(1): 1-10.
24. Widyaningrum DRW, Putri DKT, Taufiqurrahman I. Antibacterial activities of chitosan in haruan fish scales (*Channa striata*) to the growth of *Staphylococcus aureus. Dentino*. 2019; 4 (2): 163