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ANTIBACTERIAL EFFECT OF MAULI BANANA STEM EXTRACT, BASIL LEAF EXTRACT, AND THEIR COMBINATION ON *Staphylococcus aureus*

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ABSTRACT

Introduction: Mauli banana stem and basil leaf extracts have an antibacterial effect on *Staphylococcus aureus* because they contain flavonoid, saponin, alkaloid, tannin, and eugenol. 1% *povidone iodine* is commonly used as an antiseptic and gargle for recurrent aphthous stomatitis caused by *Staphylococcus aureus*. Prolonged use of 1% *povidone iodine* is notable to possess adverse effects. **Objective:** To prove the antibacterial effect of mauli banana stem extract with 25% concentration, basil leaf extract with 12.5% concentration, and mauli banana stem and basil leaf combination extract with 25%, 50%, and 75% concentration on *Staphylococcus aureus* and compared to 1% *povidone iodine*. **Methods:** This study was applying the true experimental design with post-test only and control group design which consisted of 6 treatments. Group I was given with Mauli banana stem extract with 25% concentration, group II with basil leaf extract with 12.5% concentration, group III, IV, and IV with mauli banana stem and basil leaf combination extract with 25%, 50%, and 70% concentration, respectively. Meanwhile, group VI was given with 1% *povidone iodine* as a positive control. Each treatment had 4 times repetition. Antibacterial effectivity was assessed from the inhibition zone measurement of *Staphylococcus aureus* growth using the diffusion method. **Results:** This study revealed that the mean inhibition zone formed in group I was 6-6.2 mm, group II was 6-6.2 mm, group III was 6-6.9 mm, group IV was 7.6-8 mm, group V was 8-8.5 mm, and group VI was 10.6-11.8 mm. **Conclusion:** The antibacterial efficacy of mauli banana stem extract at 25% concentration, basil leaf extract at 12.5% concentration, mauli banana and basil leaf combination extract at 25%, 50%, and 75% concentration on *Staphylococcus aureus* has been proven but not equal to 1% *povidone iodine*'s efficacy.

Keywords*: Basil leaf extract, Diffusion method, Inhibition zone, Mauli banana stem extract, Staphylococcus aureus,.*

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INTRODUCTION

The increment of health problems in Indonesia keeps raising including oral and dental health problems.[1] Recurrent aphthous stomatitis (RAS) is a painful periodic oral disease that occurs in 50% of the general population, while the prevalence of RAS reached 5% to 66% within the world population. RAS is a recurrent ulcer in the oral mucosa, known as sprue.[2,3] The etiology of RAS is not certainly known. However, *Staphylococcus aureus* has been recognized to be associated with many oral infection cases, including stomatitis.[4] *Staphylococcus aureus* may be resistant to several classes of antibiotics and conventional antiseptics. Therefore, *S. aureus* is considered as a challenging pathogen.[5,6]

Several ways of therapy had been conducted to overcome oral diseases, including the use of antiseptic mouthwash. One of the most common used antimicrobial ingredients is *povidone iodine* (PVP-I).[7] It is a widely available compound and is frequently used in clinical procedures. PVP-I is considered to have the broadest antimicrobial spectrum compared to other general antiseptics.[8] Prolonged use of PVP-I may lead to several adverse effects, including local erythema, sensitivity, pain, and mucosal erosion.[9,6]

Local plants may be utilized as an alternative medicine due to fewer adverse effects compared to chemical drugs.[10] Mauli banana (*Musa acuminata*) and basil leaf (*Ocimum sanctum* L.) are medicinal plants with many benefits.[11,12] Empirically, the upstream region of South Kalimantan, use mauli banana as a wound-healing medicine due to its antibacterial content, while Indonesian people considered basil leaf as a medicine to cure various diseases, including stomatitis.[13,14]

Mauli banana stem extract contains various substances, i.e. 14.49% saponin, 0.25% flavonoid, and 67.59% tannin. Meanwhile, the main substance contained in basil leaf is 70% eugenol, 4.6% tannin, 2% volatile oil, 1% alkaloid, and 2% flavonoid, which generate an antibacterial effect.[15,16,17] Based on a previous study by Fadhilah (2015), 25% concentration of mauli banana stem extract had the most effective antibacterial activity and was better than other concentrations against *Streptococcus mutans*.[18] This extract is known to be non-toxic to BHK-21 fibroblasts at 25% concentration.[19] Ornay (2017) proved that basil leaf extract could inhibit the growth of *Candida albicans* at 12.5% concentration.[17] The combination of tannin, saponin, and flavonoid with eugenol is known to produce a synergic effect due to different mechanisms.[20] This synergic effect enables this natural ingredient to be used as an antimicrobial agent. [21]

The use of basil leaf as a combination for mauli banana stem extract is an effort to minimalize bitterness from the banana stem. The result is obtained from the smell and the fresh effect produced by basil leaf. Machado (2012) stated that tannin in *Schinus terebinthifolius raddi* has been proven to be effective in inhibiting the growth of bacteria, and eugenol from *Syzygium aromaticum itanin* is effective in inhibiting the growth of fungi, which makes the combination of both substances produces antibacterial activity.[22,23,21] Tannin contained in mauli banana stem is known to have antiseptic and antibacterial effects, while eugenol in basil leaf is known to damage bacterial cell walls.[11,24,25] The purpose of this study was to determine the difference of antibacterial effectivity between 25% mauli banana stem extract, 12.5% basil leaf extract, and 25%, 50%, and 75% concentration of mauli banana stem and basil leaf combination extract on *Staphylococcus aureus*.

MATERIALS AND METHODS

This study was conducted after acquiring the ethical approval from the Ethical Committee of Faculty of Dentistry, ULM No. 073/KEPKG-FKGULM/EC/I/2020. This study was applying the true experimental (true laboratory) approach with post-test only and control group design, while the sampling technique used random sampling, which consisted of 6 treatment groups. Group I was given with 25% concentration of mauli banana stem extract, group II with 12.5% concentration of basil leaf extract, group III, IV, and IV with 25%, 50%, and 75% concentration of banana stem and basil leaf combination extract, and group VI as a positive control was given with 1% *povidone iodine*. The number of repetitions for each treatment was four times based on the result of the Federer formula. The population used in this study was the pure isolate of *Staphylococcus aureus* ATCC© 6538™ obtained from Airlangga University Research Center. This study was held in the Biomedical Laboratory of Faculty of Dentistry, Lambung Mangkurat University.

Extraction Procedure of Mauli Banana Stem and Basil Leaves

Mauli banana stem was obtained from Agricultural School, Banjarbaru. 12 kg of mauli banana stem was taken from a one-year-old fruitful tree. The stem was rinsed, cut into small pieces, and dried with an oven at 40-50^{o}C for 5 days. Dried mauli banana stem was mixed with a blender to obtain 600 g powder. The powder was immersed in 70% ethanol in a closed container for 3 x 24 hours. It was stirred occasionally, then filtered and let still for 4 days to precipitate dissolved substances. The extraction result was evaporated in a water bath to obtain 48.54 g of Mauli banana stem thick extract.

Basil leaves were obtained from a basil garden in Jl. Kurnia, Banjarbaru, South Kalimantan. 2 kg of basil leaves were taken following the criteria of 2 months old, bloomed, and yellow seeds were formed. The leaves were rinsed and dried at room temperature for 2 x 24 hours. Dried basil leaves were mixed in a blender to obtain 210 g of powder, which was immersed in 70% ethanol for 3 x 24 hours. The result was filtered with filter paper and remained for 3 days to precipitate dissolved substances. The extraction result was evaporated twice. The first one was performed using a rotary evaporator at 40^{o}C, then re-evaporated using a water bath to obtain 13.44 g of basil leaf thick extract.

Free ethanol test was conducted on the extraction results of mauli banana stem and basil leaf using potassium dichromate solution ($K_2Cr_2O_7$). Thick extract was diluted to form 25% concentration of mauli banana stem extract and 12.5% concentration of basil leaf extract.

Mixing Procedure of Mauli Banana Stem and Basil Leaf Extract

The main solution of mauli banana stem and basil leaf extracts with 100% concentration was obtained from a mixture of 25% concentration of mauli banana stem extract and 12.5% concentration of basil leaf extract at 1:1 volume ratio. The mixture of mauli banana stem and basil leaf extract at 100% concentration was diluted with sterile distilled water to obtain 25%, 50%, and 75% concentrations using the following formula:

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

V1 = Initial volume
C1 = Initial concentration
V2 = Final volume
C2 = Final concentration

Antibacterial Test against *Staphylococcus aureus* using Diffusion Method

Staphylococcus aureus that had been inoculated in BHI solution and adjusted according to 0.5 Mac Farland (1.5 x 10^8 CFU/ml) were smeared on an MHA media with a sterile cotton stick. A total of 0.01 ml from each of 25% mauli banana stem extract, 12.5% basil leaf extract, 25%, 50%, 75% mauli banana stem and basil leaf extract, and 1% *povidone iodine* was dripped into empty paper disks using a micropipette. The MHA media plates were incubated for 24 hours at 37^{o}C and then the inhibition zone of *Staphylococcus aureus* growth was measured with a caliper.

RESULT

The inhibition zone measurement results of Antibacterial Effectivity of Mauli Banana Stem Extract, Basil Leaf Extract, and Their Combination on *Staphylococcus* can be seen in Table 1.

Tabel 1. Inhibition Zone Measurement of Antibacterial Effectivity of Mauli Banana Stem Extract, Basil Leaf Extract, and Their Combination on *Staphylococcus aureus*

Effectivity Test of The Inhibition Zone (mm)					
K (+)	**EBPM 25%**	**EDK 12,5%**	**EKOM 25%**	**EKOM 50%**	**EKOM 75%**
10,6	6	6,1	6,9	8	8
11,8	6,1	6	6	7,8	8,2
10,8	6,2	6	6,9	7,9	8,5
11,2	6,1	6,2	6,3	7,6	8,2

Description:

EBPM 25% : 25% Mauli Banana Stem Extract
EDK 12,5% : 12,5% Basil Leaf Extract
EKOM 25% : 25% Combination Extract
EKOM 50% : 50% Combination Extract
EKOM 75% : 75% Combination Extract
K (+) : Positive Control

Table 1 represents the difference of inhibition zone between 25% concentration of mauli banana stem extract, 12.5% concentration of basil leaf extract, and 25%, 50%, and 75% concentration of mauli banana stem and basil leaf combination extract on *Staphylococcus aureus*'s growth process, which was repeated 4 times.

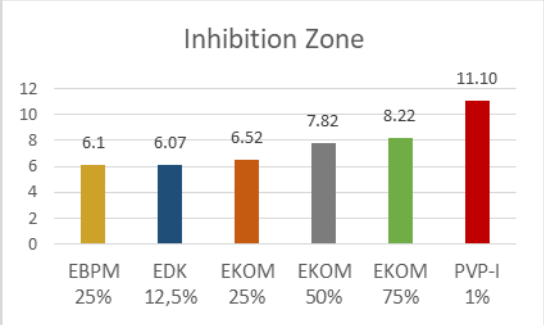

Figure 1. Bar chart depicting mean inhibition zone from each treatment against *Staphylococcus aureus*

As seen in Figure 1, the higher the extract concentration given, the bigger the inhibition zone formed. The results of this study suggested that the 75% concentration of mauli banana stem and basil leaf combination extract had a greater mean value of inhibition zone compared to 25% concentration of mauli banana stem extract, 12.5% concentration of basil leaf extract, 25% and 50% concentration of mauli banana stem and basil leaf combination extract in total of 8.22 mm. However, 75% concentration of mauli banana stem and basil leaf combination extract had a lower inhibition zone mean value compared to 1% *povidone iodine*.

The obtained data from each treatment were tabulated and subjected for normality analysis using

Shapiro-Wilk test and was considered normal if $p > 0.05$. The normality test result of 25% concentration of mauli banana stem extract, 12.5% concentration of basil leaf extract, 25%, 50%, and 75% concentration of mauli banana stem and basil leaf combination extract and 1% *povidone iodine* on *Staphylococcus aureus* showed $p > 0.05$, thus the data were normally distributed. The data then got submitted to a homogeneity analysis using Levene's test and obtained a significance value of 0.012 ($p < 0.05$), which means that the data were not homogenous. The result of One-Way ANOVA revealed 0.000 ($p < 0.05$), which explained that several groups had significant differences. Therefore, the Games-Howell Post-Hoc test was required to determine which groups had significant differences.

Games-Howell Post-Hoc test revealed that 25% concentration of mauli banana stem extract, 12,5% concentration of basil leaf extract, and 25% concentration of mauli banana stem and basil leaf combination extract had $p > 0.05$, which means that the inhibition zone diameter difference between these concentrations were statistically insignificant. Meanwhile, 50% and 75% concentration of mauli banana stem and basil leaf combination extract and 1% *povidone iodine* extract revealed $p < 0.05$, which means that the inhibition zone diameter between these concentrations were statistically significant.

DISCUSSION

Based on the result of this study, 25% concentration of mauli banana stem and 12.5% concentration of basil leaf extract produced inhibition zone of 6.-6.2 mm, 25% concentration of mauli banana stem and basil leaf combination extract produced 6-6.9 mm, 50% concentration of mauli banana stem and basil leaf combination extract produced 7.6-8 mm, and 75% concentration of mauli banana stem and basil leaf combination extract produced 8-8.5 mm inhibition zone. This was in line with Apriasari (2013), who indicated that 25% concentration of mauli banana stem extract was sufficient in inhibiting the growth of *Streptococcus mutans* and Ornay (2017) who proved that basil leaf extract could inhibit the growth of *Candida albicans* with 12.5% concentration.[18,17] The 75% concentration of mauli banana stem and basil leaf combination extract in inhibiting the growth of *Staphylococcus aureus* produced greater inhibition zone than 25% concentration of mauli banana stem extract, 12.5% basil leaf extract, 25% and 50% concentration of mauli banana stem and basil leaf combination extract. This was convenient with Noor and Apriasari (2014) statement that 25%, 80%, and 100% concentration of mauli banana stem extract produced an average inhibition zone against *Streptococcus mutans* with a diameter of 11.17 mm, 15 mm, and 16.17 mm. This study also exposed that 100% mauli banana stem extract had an optimal result in suppressing the growth of *Streptococcus mutans* compared to other concentration.[13] Hamad (2017) stated that a higher concentration might produce an increase in osmotic pressure which caused the intracell liquid from bacteria cells into the lower concentration. This condition would induce bacteria cell contraction that leads to cell death.[26]

Mauli banana stem and basil leaf combination extract at 75% concentration indicated lower inhibition zone compared to 1% *povidone iodine* in inhibiting the growth of *Staphylococcus aureus* because of *povidone iodine* antibacterial mechanism is by changing the structure and function of cell protein and enzyme, and inhibiting bacteria's hydrogen attachment. It changes the structure of the cell membrane and acts in inhibiting protein synthesis through thiol oxidation process in cysteine amino acid.[7] The results showed that the combination of mauli banana stem and basil leaf extract increased antibacterial inhibitory ability on the growth of *Staphylococcus aureus* compared to single extract. This means that there was a synergic activity between the active substances contained in both extracts.[20] Sukandar *et al* (2014) proposed a theory that a combination of extracts is synergistic if they produced greater antibacterial activity with smaller concentration compared to single extract.[27]

Active substances contained in mauli banana stem and basil leaf indicated substantial bacterial activities. Substances that has role as antibacterial agents in mauli banana stem are polyphenols and other phenolic substances, including their derivates which may cause protein denaturation.[18] Flavonoid acts by denaturing bacterial cell protein and damaging cytoplasm, which leads to a leakage of important metabolites and inactivated bacterial enzyme system, enabling nucleotide and amino acids to seep out. Tannin inhibits the growth of *Staphylococcus aureus* by inactivating the adhesion of microbial cells (molecules attached to host cells) contained in cell surfaces.[19] Saponin works by inhibiting bacterial protein synthesis, causing changes in the components forming bacterial cells.[18] Eugenol in basil leaf damages cell wall in *Staphylococcus aureus*, thus causing lysis which leads protein, lipid, potassium, and ATP to escape cell walls and inhibiting bacterial growth.[25] Another content that has an antibacterial effect is alkaloid. It works by affecting cell division, inhibiting respiration and bacterial enzyme, causing disruption to cell membrane that affects gene virulence. The role of

alkaloid in inhibiting the growth of bacteria is also associated with its ability to intercalate with DNA, thus inhibiting DNA synthesis.[28,29]

Based on the statements above, the antibacterial efficacy of mauli banana stem extract at 25% concentration, basil leaf extract at 12.5% concentration, mauli banana and basil leaf combination extract at 25%, 50%, and 75% concentrations has been proven but not equal to 1% *povidone iodine*'s efficacy. The increment of inhibition zone is evident from 50% concentration of the combination of mauli banana stem and basil leaf and keep increasing along with higher concentration. Therefore, further studies may use higher concentrations and different volume ratios from each extract for a better result.

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