

DENTINO
JURNAL KEDOKTERAN GIGI
Vol V. No 2. September 2020

ANTIFUNGAL EFFECT OF MAULI BANANA STEM EXTRACT, BASIL LEAF EXTRACT, AND THEIR COMBINATION ON *Candida albicans*

Hadi Waskito, Maharani Laillyza Apriasari², Juliyatin Putri Utami³

¹ Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

² Department of Oral Medicine Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

³ Department of Biomedics Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

ABSTRACT

Backgrounds: Oral candidiasis is an oral infection caused by abnormal growth of *Candida albicans*. The use of 0.2% *chlorhexidine gluconate* as a prophylactic mouthwash is conducted for oral candidiasis therapy. Prolonged use of 0.2% *chlorhexidine gluconate* is recorded to instigate side effects. Mauli banana stem extract and basil leaf extract demonstrate antifungal properties ascribed to their contents. **Objective:** To prove that the antifungal effect of 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 *Candida albicans* are comparable to 0.2% *chlorhexidine gluconate*. **Methods:** This study was resolved by applying the true experimental design with post-test only and control group design which consisted of 6 treatments. Group I was given Mauli banana stem extract with 25% concentration, group II was given basil leaf extract with 12.5% concentration, group III, IV, and IV were given the combination of Mauli banana stem and basil leaf extracts with 25%, 50%, and 75% concentrations, respectively. Meanwhile, group VI was given 1% *chlorhexidine gluconate* as a positive control. Each treatment was served with four times repetition. Antibacterial effectivity was assessed through the inhibition zone measurement of 0.2% *chlorhexidine gluconate* growth using the diffusion method. **Results:** This study revealed that the average diameter of inhibition zone formed in group I was 6-6.3 mm, group II was 6-6.2 mm, group III was 6-6.2 mm, group IV was 7-7.2 mm, group V was 10.2-11.4 mm, and group VI was 23.7-24.8 mm. **Conclusion:** The antifungal effect of mauli banana stem extract at 25% concentration, basil leaf extract at 12.5% concentration, and mauli banana stem and basil leaf combination extract at 25%, 50%, and 75% concentration on *Candida albicans* has been proven but not equivalent to 0.2% *chlorhexidine gluconate*.

Keywords: *Basil leaf extract, Candida albicans, inhibition zone, 0.2% chlorhexidine gluconate, diffusion method, Mauli banana stem extract.*

Correspondence: Hadi Waskito, Faculty of Dentistry, Lambung Mangkurat University, Jl. Veteran 128B Banjarmasin, South Kalimantan, email: hadiwask@gmail.com

INTRODUCTION

Oral candidiasis is an oral infection caused by abnormal growth of *Candida albicans*.¹ Oral candidiasis is placed on the third position within dermatomycosis incidence in Indonesia, with a quite high mortality rate of 64.8%, similar to a study in Brazil which showed a total of 54%.² This disease could result in oral discomfort, dysphagia, taste sensation disorder, and malnutrition.³ It can be caused by several factors, including systemic diseases such as diabetes mellitus, immunosuppressive condition, HIV, chemotherapy, malignancy such as leukemia, smoking habit, the use of dentures, xerostomia, and long-term medication

such as antibiotics.⁴

Prolonged use of antifungal drugs could cause several side effects such as a burning sensation due to irritation from nystatin and miconazole. Prolonged use of 0.2% *chlorhexidine gluconate* will also generate discoloration of the teeth and loss of taste, as well as an increase in fungal resistance toward antifungal therapy.^{5,6} The stimulus to *Candida* resistance is long-term use of antifungal drugs, thus the need to search for new potential ingredients emerged.⁵

Traditional medicines believed by the community as natural ingredients with the potentials

for the treatment of fungal infection are Mauli banana stem and basil leaf.^{7,8} The bioactive contents in Mauli banana stem are tannin with 67.59%, saponin 14.49%, alkaloid 3.44%, and flavonoid 0.25%. Meanwhile, the main substances in basil leaves are eugenol with 70%, tannin 4.6%, volatile oil 2%, flavonoid 2%, triterpenoid 2%, steroid 2%, and alkaloid 1%.^{9,10,11} Combination of tannin, saponin, flavonoid, and alkaloid with eugenol has been proven to produce a synergic effect due to different mechanisms between each substance.¹² This synergic effect is beneficial for this natural ingredient to be used as an antimicrobial agent.¹³ Combining basil leaves to the mixture becomes an adequate choice, because basil has an antifungal effect and produces a fresh and well-scented feeling, which can minimize the bitter taste of Mauli banana stem.¹⁴

The purpose of this study was to determine the antifungal effectivity of Mauli banana stem single extract with 25% concentration, basil leaf single extract with 12.5% concentration, and combination of Mauli banana stem and basil leaf extracts with 25%, 50%, and 75% concentrations, compared to 0.2% *chlorhexidine gluconate* on the growth of *Candida albicans*.

MATERIALS AND METHODS

This study has received ethical approval from The Ethical Committee of the ULM Faculty of Dentistry No. 106/KEPKG-FKGULM/EC/II/2020. The design used in this study was true experimental with a post-test only with control group design and the samples were chosen using random sampling, which consisted of 6 treatment groups. Group I was given 25% Mauli banana stem single extract. Group II was given 12.5% basil leaf single extract. Group III was given a 25% combination of Mauli banana stem and basil leaf extracts. Group IV was given a 50% combination of Mauli banana stem and basil leaf extracts. Group V was given a 75% combination of Mauli banana stem and basil leaf extracts. Meanwhile, Group VI was given 0.2% *chlorhexidine gluconate*. Each treatment was repeated 4 times following the Federer formula. The population used in this study was a pure isolate of *Candida albicans* ATCC® 10231™ obtained from PT. EMBRIO Biotekindo Laboratory.

Extraction Procedure of Mauli Banana Stem

Mauli banana stem was obtained from *Sekolah Pertanian Pembangunan Negeri* Banjarbaru, South Kalimantan. Five kilogram of Mauli banana stem was taken from a one-year-old fruiting tree. The stem was rinsed and cut into small pieces, then dried in an oven at 40-50°C for 5 days. Dried Mauli banana stem

was crushed using a blender to make a 600 g powder. The powder was immersed in 70% ethanol in a closed container for 3 x 24 hours, stirred occasionally and filtered, then remained for 4 days to precipitate dissolved substances. The extraction result was evaporated in a rotary evaporator at 40°C and re-evaporated using a waterbath to obtain 48.54 g of Mauli banana stem thick extract.

Extraction Procedure of Basil Leaves

Basil leaves were obtained from a basil garden located at Jl. Kurnia, Banjarbaru, South Kalimantan. Basil leaves, with a total weight of 1.5 kg, were chosen according to the following criteria, i.e. aged 2 months, fully blossomed, and yellow-coloured on the lower part of the leaves. The leaves were rinsed and dried at room temperature for 2 x 24 hours. Dried basil leaves were blended to obtain 210 g of powder. The powder was immersed in 70% ethanol for 3 days, stirred occasionally, and filtered. Afterward, the powder remained for 3 days to precipitate dissolved substances. The extraction result was evaporated 2 times, the first one using a rotary evaporator at 40°C, then the second one using waterbath to obtain 13.44 g thick basil leaf extract.

Free Ethanol Test

Mauli banana stem extract, basil leaf extract, and mauli banana stem and basil leaf combination extract was subjected to free ethanol test using potassium dichromate solution ($K_2Cr_2O_7$). Mauli banana stem extract with 25% concentration and 12% basil leaf extract were obtained from diluting 100% Mauli banana leaf and basil leaf extracts.

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% mauli banana stem extract and 12.5% basil leaf extract with a 1:1 volume ratio. The mixture of mauli banana stem and basil leaf extract with 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

Candida albicans Sample Culture

Several colonies of *Candida albicans* ATCC® 10231™ was inoculated on the SDA media. The media was put into an anaerobic incubator to incubate the colonies for 1 x 24 hours at 37°C. The solution of 0.5 ml *Candida* was inoculated into 5 ml of liquid BHI and incubated for 2 x 24 hours at 37°C in an anaerobic incubator. The suspension was diluted with BHI liquid media to obtain turbidity equal to 0.5 McFarland standard, or equal to 1.5 x 10⁸ CFU.

Antifungal Effectivity Test

The *Candida albicans* ATCC® 10231™ equal to 0.5 McFarland standard was smeared with a cotton swab on the MHA testing media. A total of 0.01 ml from each of 25% Mauli banana stem, 12.5% basil leaf, 25%, 50%, 75% combination of Mauli banana stem and basil leaf extracts, and 0.2% *chlorhexidine gluconate* was dropped into empty paper disks using a micropipette. MHA was incubated at 37°C for 24 hours. Each fungal inhibition zone was measured with a caliper.

RESULTS & ANALYSIS

Measurement results of inhibition zone diameter from each treatment on the growth of *Candida albicans* can be seen in the figures and tables below.

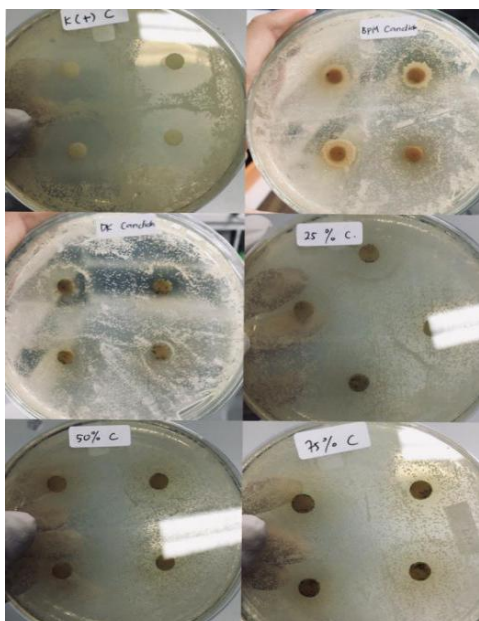


Figure 1. (A) Inhibition zone of 0.2% *chlorhexidine gluconate*; (B) Inhibition zone of 25% Mauli banana stem single extract; (C) Inhibition zone of 12.5% basil leaf single extract; (D) Inhibition zone of 25% combination extract; (E) Inhibition zone of 50%

combination extract; (F) Inhibition zone of 75% combination extract on *Candida albicans* with 4 times repetition.

Table 1. Measurement results of inhibition zones of Mauli banana stem extract, basil leaf extract, combination extract, and positive control on the growth of *Candida albicans*.

Effectivity Test of The Inhibition Zone					
K (+)	EBPM	EDK	EKOM	EKOM	EKOM
	25%	12,5%	25%	50%	75%
23,7	6,1	6,1	6,2	7	11,4
24,4	6,2	6	6,1	7,1	10,2
24,8	6,3	6,2	6	7,2	10,8
24,7	6,1	6	6,1	7,1	11,1

Table 1 showed that there were differences in inhibition zone diameter between 25% Mauli banana stem single extract and 12.5% basil leaf single extract, 25%, 50%, 75% combination extract, and positive control on the growth of *Candida albicans* ATCC® 10231™

Antifungal effectivity was tested using the measurement of inhibition zone diameter, which is a transparent zone surrounding the paper disks of each sample from each treatment group using a caliper in mm. Subsequently, the data were processed using IBM SPSS Statistics 26 for Windows to receive the following results.

Table 2. Mean inhibition zone of mauli banana stem extract, basil leaf extract, combination extract, and positive control on the growth of *Candida albicans*.

Treatments	N	Mean	Std. Deviation
EBPM 25%	4	6,17	0,04
EDK 12,5%	4	6,07	0,04
EKOM 25%	4	6,10	0,04
EKOM 50%	4	7,10	0,04
EKOM 75%	4	10,87	0,25
CHX 0,2%	4	24,40	0,24

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

CHX 0.2% : 0.2% *Chlorhexidine gluconate*

Table 2 showed the mean and standard deviation (SD) of each treatment group. Mauli banana stem extract with 25% concentration showed

a mean value of 6.17 mm and 0.04 SD. Basil leaf extract with 12.5% concentration showed a mean value of 6.07 mm and 0.04 SD. Combination extract with 25% concentration showed a mean value of 6.10 mm and 0.04 SD. Combination extract with 50% concentration showed a mean value of 7.10 mm and 0.04 SD. Combination extract with 25% concentration showed a mean value of 10.87 mm and 0.25 SD. *Chlorhexidine gluconate* with 0.2% concentration showed a mean value of 24.40 mm and 0.24 SD.

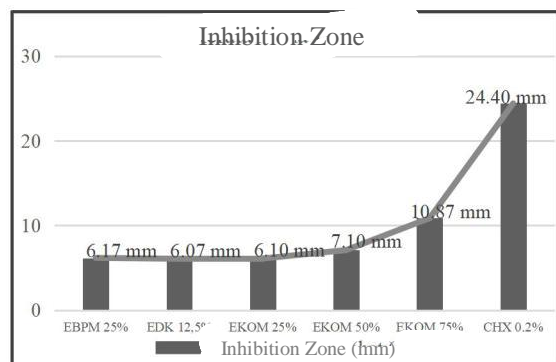


Figure 2. Bar chart depicting the inhibition zones of Mauli banana stem extract, basil leaf extract, combination extract, and positive control on the growth of *Candida albicans*.

Figure 2 indicated that 50% combination extract started to show an increase in inhibition zone compared to 25% Mauli banana stem extract, 12.5% basil leaf extract, and 25% combination extract. Based on the results of this study, the combination extract of Mauli banana stem and basil leaf with 75% concentration had a higher inhibition zone compared to the other groups with a value of 10.87 mm, except for 0.2% *chlorhexidine gluconate*, which had a higher mean of inhibition zone.

Table 3. Shapiro-Wilk normality test on the inhibition zone of Mauli banana stem extract, basil leaf extract, combination extract, and positive control on the growth of *Candida albicans*.

Group	df	Sig.
EBPM 25%	4	0.272
EDK 12,5%	4	0.272
EKOM 25%	4	0.683
EKOM 50%	4	0.683
EKOM 75%	4	0.850
CHX 0,2%	4	0,329

Table 3 revealed a normally distributed data of Mauli banana stem extract, basil leaf extract, combination extract, and positive control on the

growth of *Candida albicans* with $p > 0.05$. The data were subjected to homogeneity testing using Levene's test and showed a significance value of 0.023 ($p < 0.05$). Then, One-Way ANOVA was performed and revealed a p-value of 0.000 ($p < 0.05$), which showed several groups with significant differences. Games-Howell Post Hoc test was used to determine which groups differed significantly with the following results.

Table 4. Games-Howell Post Hoc Test on the inhibition zone of Mauli banana stem extract, basil leaf extract, combination extract, and positive control on the growth of *Candida albicans*.

	EBPM 25%	EDK 12,5%	EKOM 25%	EKOM 50%	EKOM 75%
EBPM 25%		.689	.827	.000*	.001*
EDK 12,5%			.998	.000*	.001*
EKOM 25%				.000*	.001*
EKOM 50%					.003*
EKOM 75%					
CHX 0,2%	.000*	.000*	.000*	.000*	.000*

*significance ($p < 0,05$)

The results of Games Howell Post Hoc test in Table 4 indicated that 25% Mauli banana stem extract, 12.5% basil leaf extract, and 25% combination extract had $p > 0.05$, which showed statistically insignificant difference compared to 50% combination extract, 75% combination extract, and 0.2% *chlorhexidine gluconate*. Meanwhile, 50% combination extract, 75% combination extract, and 0.2% *chlorhexidine gluconate* showed $p < 0.05$, which means that the inhibition zone concentrations between the groups were statistically significant.

DISCUSSION

This study used the diffusion method to analyze the antifungal inhibition zone of the ingredients by measuring inhibition zone diameter from 25% Mauli banana stem extract, 12.5% basil leaf extract, 25%, 50%, 75% combination extract, and positive control on the growth of *Candida albicans*. The inhibition zone was measured to determine the antifungal effect from mauli banana stem extract at 25% concentration, basil leaf extract at 12,5% concentration, and mauli banana and basil

leaf combination extract at 25%, 50%, 75% concentration to obtain extract concentration with equal antifungal effectivity to the positive control on the growth of *Candida albicans*.

Mauli banana stem extract with 25% concentration produced 6-6.3 mm inhibition zone, 12.5% basil leaf and 25% combination extract produced 6-6.2 mm inhibition zone, 50% combination extract produced 7-7.2 mm inhibition zone, 75% combination extract produced 10.2-11.4 mm inhibition zone.

Apriasari (2015) had proven that 25% of Mauli banana stem extract was effective in inhibiting the growth of *Candida albicans*. Ornay (2017) proved that basil leaf extract can inhibit the growth of *Candida albicans* at 12.5% concentration. Increased inhibition zone was started from 50% combination extract and kept increasing along with concentration increase. Combination extract of mauli banana stem and basil leaf at 75% produces higher inhibition zone on the growth of *Candida albicans* compared to 25% Mauli banana stem extract, 12.5% basil leaf extract, 25% and 50% combination extract of Mauli banana stem and basil leaf.

Apriasari (2015) proved that 100% Mauli banana stem extract had the optimal result on suppressing the growth of *Candida albicans* compared to Mauli banana stem with 25% and 80% concentration, which means that higher concentration will produce higher content of secondary metabolites.^{7,8}

Sukandar *et al.* (2014) expressed a theory that a combination extract is synergistic if it gives higher antifungal effectivity with lower concentration compared to single concentration.¹⁵ Combination extract of Mauli banana stem and basil leaf with 75% concentration showed a lower inhibition zone than 0.2% *chlorhexidine gluconate* in inhibiting the growth of *Candida albicans*. This was in line with Eggers (2019), who proposed that 0.2% *chlorhexidine gluconate* was effective in reducing the colonization of *Candida albicans* because of the active antiseptic activity of bisguanide, which can act as a broad-spectrum antiseptic to inhibit the growth of microorganisms. The mechanism of *chlorhexidine gluconate* as an antifungal agent is by damaging cell membranes through the insertion of amphiphilic molecules owned by *chlorhexidine* into cell membranes, attacking the plasma membrane in fungi. Chlorhexidine can reduce the ability of *candida* adherence to host cells, and it can attach to glycoproteins in saliva and released periodically (Apriasari, 2015).^{7,16}

The active substances with a substantial antimicrobial activity responsible for the antifungal effect of Mauli banana stem and basil leaf were included in the polyphenol group and other phenolic substances which can damage fungal cells. Flavonoid is an active substance contained in Mauli banana stem and basil leaf, which is a phenolic substance acts as an antifungal agent with the working mechanism of damaging cell membrane and disrupting the mitochondrial function of the fungal cells. Alkaloid mechanism as an antifungal agent is by inhibiting the proliferation of protein formation, disrupting cell respiration, and causing fungal cell death.^{7,8,14}

Mauli banana stem and basil leaf also have saponin and tannin as its content, which exhibit antifungal activities. Saponin works by reducing stress on the sterol membrane during the synthesis process of fungal cell walls. Tannin is a complex polyphenol substance that can inhibit chitin cell synthesis, which is an important component of *Candida albicans* by reacting with cell walls. Flavonoid, alkaloid, saponin, and tannin will interact with fungal cell surface through a hydrogen bond.⁶ The most common substance in basil leaves is eugenol. The interaction between eugenol and fungal cell occurs in the absorption process involving hydrogen bond, followed by penetration into the cells, causing precipitation and denaturation of proteins. The phenol protein complex formed at a low level with a weak bond will disintegrate.⁸

The inhibition zone results of 25% Mauli banana stem, 12.5% basil leaf, and 25%, 50%, and 75% combination extracts were unable to surpass 0.2% *chlorhexidine gluconate*. Further studies with higher concentrations and different volume ratios are needed to obtain higher inhibition zone.

REFERENCES

1. Kusumaputra BH, Zulkarnain I. Penatalaksanaan Kandidiasis Mukokutan pada Bayi. *Periodical of Dermatology and Venerology*. 2014 Agustus; 26(2): 139-145.
2. Kalista KF, Chen LK, Wahyuningsih R, Rumende CM. Karakteristik Klinis dan Prevalensi pasien Kandidiasis Invasif di Rumah Sakit Cipto Mangunkusumo. *Jurnal Penyakit Dalam Indonesia*. 2017 Juni; 4(2): 56-61.
3. Mubarak Z, Gani BA, Mutia. Daya Hambat Kunit (*Curcuma longa Linn*) Terhadap Pertumbuhan *Candia albicans*. *Cakradokya Dental Journal*. 2019; 11(1): 1-7.
4. Mutiawati VK. Pemeriksaan Mikrobiologi pada *Candida albicans*. *Jurnal Kedokteran Syiah kuala*. 2016 Agustus; 16(1): 53-62.

5. Dias II, Trajano ERIS, Castro RD, Ferreira GLS, Medeiros HCM, Gomes DQC. Antifungal Activity of Linalool In Cases of *Candida* spp. Isolated From Individuals with Oral Candidiasis. *Brazilian Journal of Biology*. 2016 October; 368-374.
6. Permatasari, Budiarti LY, Apriasari ML. 2016. Efektivitas Antifungi Ekstrak Metanol Batang Pisang Mauli (*Musa Acuminata*) dan Chlorhexidine Gluconate 0,2% Terhadap *Candida albicans*. *Dentino (Jurnal Kedokteran Gigi)*. 1(1): 10 – 14.
7. Apriasari ML, Iskandar, Suhartono E. Antibacterial activity and total flavonoid of mauli banana stem. *IPCBE*. 2015; 83 (27): 153.
8. Ornay AKD, Prehananto H, Dewi ASS. 2017. Growth Inhibition of *Candida albicans* And Power Kill *Candida albicans* Extract Basil Leave. *Jurnal Wiyata*. 4(1): 78-83.
9. 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 Pharmaceutics*. 2019; 11 (1): 184.
10. Puspitasari D, Apriasari ML. Effect of 25%, 37,5%, 50% Mauli Banana (*Musa acuminata*) Stem Extract as Mouthwash on Color Stability of Bulk Fill Resin Composite. *Dentino Journal*. 2018 September; 3(2): 168-173.
11. El-baky RMA, Hashem ZS. Eugenol and Linalool: comparison of their antibacterial and antifungal activity. 2016; 10 (44): 32.
12. Safirah R, Widodo N, Budiyanto MAK. Uji Efektifitas Insektisida Nabati Buah *Crescentia cujete* dan Bunga *Syzygium aromaticum* terhadap Moralitas Spodoptera litura secara In Vitro sebagai Sumber Belajar Biologi. *Jurnal Pendidikan Biologi Indonesia*. 2016; 2(3): 265-276.
13. 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.
14. Saputri MV, Carabelly AN, Firdaus IWAK. Toxicity test of the mixed mouthwash ash of mauli banana stem and basil leaf against fibroblast cell study in vitro. *Dentino*. 2019; 4 (2): 151-152.
15. 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.
16. Eggers M. Infectious Disease Management and Control with Povidone iodine. *Adis Journal*. 2019 Agustus; 581-593.