

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

**ANTIFUNGAL ACTIVITIES OF MAULI BANANA STEM AND BASIL LEAVES  
EXTRACT MIXTURE AGAINST *Candida albicans***

Nor Kamalia<sup>1</sup>, Maharani Laillyza Apriasari<sup>2</sup>, Yusrinie Wasiaturrahmah<sup>3</sup>

<sup>1</sup> Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

<sup>2</sup> Department of Oral Medicine, Faculty of Dentistry, Lambung Mangkurat, Banjarmasin

<sup>3</sup> Department of Biomedic, Faculty of Dentistry, Lambung Mangkurat University, Banjarmasin

**ABSTRACT**

**Background:** *Mauli banana stem and basil leaves are natural ingredients that have the potential to be used as a treatment for Candida albicans infections. The methanol extract of mauli banana stem with 25% concentration is merely fungistatic which is still not capable to induce fungicidal effect, while the 100% concentration is known to be less effective than 0.2% chlorhexidine gluconate. Adding basil leaves extract to mauli banana stem extract is expected to increase antifungal activities against Candida albicans. Purpose:* to analyze the antifungal activity of mauli banana stem and basil leaves extract mixture with concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% against *Candida albicans* by dilution method. **Method:** This research used a true experimental method with a pretest–posttest control group design consisting of 11 treatments to obtain Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). **Result:** The 10% concentration was identified as the Minimum Inhibitory Concentration (MIC), and no concentration could be classified as the Minimum Bactericidal Concentration (MBC) because all treatment group still exhibited *Candida albicans* growth on the SDA medium. **Conclusion:** *Mauli banana stem and basil leaves extract mixture has an MIC value of 10% and does not demonstrate any value for the MBC.*

**Keywords:** *Basil leaf, Candida albicans, Mauli banana stem, MBC, MIC*

**Correspondence:** Nor Kamalia, Faculty of Dentistry, University of Lambung Mangkurat, Veteran Street No.128 B Banjarmasin, Indonesia. Email: [norkamalia3@gmail.com](mailto:norkamalia3@gmail.com)

**INTRODUCTION**

*Candida albicans* is part of the normal flora in the oral cavity which often causes *oral candidiasis*.<sup>1,2</sup> Oral candidiasis in Indonesia occurred approximately 7,098 cases in 2012 and ranked as the third most common diseases in RSGM Gusti Hasan Aman Banjarmasin, South Kalimantan from 2014-2017.<sup>3,4</sup> Prolonged use of antifungal drugs may trigger *Candida albicans* resistance. The emergence of *Candida albicans* resistance encourages the search for natural ingredients that may be utilized as alternative medicines in the treatment of *Candida albicans* infections.<sup>5</sup>

Natural ingredient that has the potential to be used as a treatment for *Candida albicans* infection is the stem of mauli banana. The bioactive ingredients in the Mauli banana stem are tannins 67.9%, saponins 14.49%, and flavonoids 0.25% which function as antifungal compounds.<sup>6</sup> Methanol extract of mauli banana stem at a 25% concentration may inhibit *Candida albicans* but is still not capable of being fungicidal, while the 100% concentration is known to be less effective than 0.2% *chlorhexidine gluconate*. Methanol

extract of mauli banana stem 100% could only inhibit *Candida albicans* by 19 mm while 0.2% *chlorhexidine gluconate* by 21 mm.<sup>3,7</sup>

The aims of basil leaves extract addition to mauli banana stem extract is to increase the antifungal activity against *Candida albicans* because it has bioactive content such as eugenols, flavonoids, essential oils, tannins, and alkaloids which function as antifungal compounds. Basil leaves extract with 12.5% concentration may inhibit the growth of *Candida albicans* and the 25% concentration may kill *Candida albicans*.<sup>8</sup> The addition of basil leaves extract may also provide a fresh effect and fragrant aroma so that it can neutralize the bitter taste prompted by the extract of Mauli banana stem.<sup>9</sup>

Based on that, this research aims to analyze the antifungal activity of Mauli banana stem and basil leaves extract mixture at 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100% concentrations against *Candida albicans* by dilution method.

## MATERIALS AND METHOD

This study was initiated by collecting research permit and ethical clearance issued by the Ethics Committee of Faculty of Dentistry, Lambung Mangkurat University No. 073 / KEPKG-FKGULM / EC / I / 2020. This research used a true experimental method with a *pretest–posttest control group design* consisting of 11 treatments, those are concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, and negative controls in the form of aquadest. The minimum number of repetitions for each treatment were 3 times based on Federer formula. The population used are pure isolate of *Candida albicans* ATCC 10231 obtained from the MBRIO food laboratory Bogor.

### Mauli Banana Stem Extraction

A total of 12 kg mauli banana stem was taken from a one-year-old tree that has been bearing fruit. The tree was located at the State Vocational High School of Agriculture and Development Banjarbaru city where the stem was going to be obtained. Mauli banana stem was washed, cut into small pieces and dried in an oven at 40-50°C for 5 days. Mauli banana stem that had been dried was then mashed using a blender until it became a simplicia powder and obtained the weight of 600 g. Simplicia powder of mauli banana stem was soaked in 70% ethanol for 3x24 hours while occasionally stirred and strained to be further precipitated for 4 days. The extraction result was evaporated using a rotary evaporator at 40°C and re-evaporated using a waterbath until as much as 48.54 g thick extract of mauli banana stem was obtained. The result of mauli banana extract was proceeded to ethanol-free testing using *potassium dichromate* (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>). Mauli banana extract used in this study was a concentration of 25%, which was obtained by diluting the mauli banana thick extract of 100% concentration.

### Basil Leaves Extraction

A total of 2 kg basil leaves aged two months old was taken from Jl. Kurnia, Banjarbaru, South Kalimantan. Basil leaves were washed and dried indoor for 48 hours. Dried Basil leaves were then mashed using a blender until it became a simplicia powder and obtained the weight of 210 g. Simplicia powder of basil leaves was soaked with 70% ethanol for 3x24 hours while it was occasionally stirred and was strained to further be precipitated for 3 days. The extraction result was evaporated using a rotary evaporator at 40°C and re-evaporated using a waterbath until as much as 13.44 g thick extract of basil leaves was obtained. The result of basil leaves extract was proceeded ethanol-free testing using *potassium dichromate* (K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>). Basil leaves extract used in this study was a

concentration of 12,5%, which was obtained by diluting the basil leaves thick extract of 100% concentration.

### Mixing Mauli Banana Stem Extract and Basil Leaves Extract

The mother liquor of mauli banana stem extract and basil leaves extract mixture with a 100% concentration is a combination from 25% mauli banana stem extract and 12.5% basil leaves extract with 1:1 volume ratio. A solution of mauli banana stem extract and basil leaves extract mixture at the concentration of 100% was diluted using sterile aquadest in various concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%.

### Cultivating *Candida albicans* Samples

The pure isolate of *Candida albicans* was inoculated to the SDA media and incubated for 24 hours at 37°C and aerobic condition. *Candida albicans* that had grown was inoculated using inoculating loop into the BHI-B media and diluted by adding aquadest until the turbidity was comparable to the 0.5 *McFarland* standard (1.5x10<sup>8</sup> CFU/ml).

### Minimum Inhibitory Concentration (MIC) Testing

The mauli banana stem and basil leaves extract mixture that had been added with *Candida albicans* suspension in each treatment was observed to measure the initial absorbance value using a spectrophotometer Biobase B-KD 560 ( $\lambda = 600$  nm). After obtaining the initial absorbance value then it was incubated for 24 hours at 37°C and aerobic conditions. After the incubation, the final absorbance value was measured using a spectrophotometer Biobase B-KD 560 ( $\lambda = 600$  nm).

### Minimum Bactericidal Concentration (MBC) Testing

A total of 5  $\mu$ L from each treatment was inoculated into *Sabouraud Dextrose Agar* media after the MIC value was obtained and incubated under aerobic conditions for 24 hours at 37°C temperature. The number of colonies were calculated using *colony counter* to determine the MBC value. If the result is 0, it means there is no fungus or spots on the SDA media, thus the MBC value is obtained.

### Result

The Minimum Inhibitory Concentration (MIC) of mauli banana stem and basil leaves extract mixture against *Candida albicans* obtained results as presented in Table 1 below.

**Table 1. Minimum Inhibitory Concentration (MIC) Result of Mauli Banana Stem and Basil Leaves Extract Mixture Against *Candida albicans***

Group	N	Incubation (0 hours)	Incubation (24 hours)	Difference	Information	Mean ± Standard Deviation
10%	3	0.167	0.146	-0.021	Down	-0.02100 ± 0.010536
20%	3	0.220	0.189	-0.031	Down	-0.03167 ± 0.012342
30%	3	0.369	0.233	-0.136	Down	-0.13600 ± 0.013000
40%	3	0.493	0.319	-0.174	Down	-0.17367 ± 0.011240
50%	3	0.633	0.459	-0.174	Down	-0.17467 ± 0.041489
60%	3	0.709	0.599	-0.11	Down	-0.11000 ± 0.004583
70%	3	0.921	0.811	-0.11	Down	-0.11033 ± 0.009504
80%	3	1.145	0.967	-0.178	Down	-0.17833 ± 0.023159
90%	3	1.345	1.142	-0.203	Down	-0.20267 ± 0.003215
100%	3	1.483	1.266	-0.217	Down	-0.21767 ± 0.006658
Control (-)	3	0.549	0.849	0,3	Up	0.30033 ± 0.025423

Table 1 shows that 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% concentrations of mauli banana stem and basil leaves extract mixture were able to inhibit the growth of *Candida albicans*. This is indicated by the decrease of absorbance value after the incubation. Based on these results, the 10% concentration was determined as the Minimum Inhibitory Concentration (MIC).

MIC data that has been obtained from each treatment was examined for normality test using *Shapiro-wilk* and obtained  $p > 0.05$  which means

that the data was normally distributed. Next, the homogeneity test was performed using the *Levene's test*. The result of *Levene's test* shows that the significance value was less than 0.05 ( $p = 0.011$ ) which means the data was not homogeneous. *One Way ANOVA* analysis results obtained a significance value of  $p = 0.000$  ( $p < 0.05$ ), which means that there were significant differences of average between each treatment group. The data analysis was continued with the *Post Hoc Games-Howell test* in Table 2.

**Table 2. Post Hoc Games-Howell Test Results of Minimum Inhibitory Concentration (MIC) of Mauli Banana Stem and Basil Leaves Extract Mixture Against *Candida albicans***

Group	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%	Control (-)
10%		0.962	0.004*	0.001*	0.106	0.011*	0.005*	0.018*	0.003*	0.000*	0.004*
20%			0.006*	0.002*	0.119	0.026*	0.013*	0.018*	0.006*	0.001*	0.003*
30%				0.179	0.839	0.339	0.398	0.417	0.054	0.019*	0.001*
40%					1.000	0.032*	0.021*	1.000	0.214	0.068	0.001*
50%						0.491	0.498	1.000	0.939	0.760	0.002*
60%							1.000	0.172	0.000*	0.001*	0.005*
70%								0.155	0.010*	0.002*	0.003*
80%									0.750	0.440	0.000*
90%										0.271	0.004*
100%											0.002*
Control (-)											

Information :

\* = significant difference ( $p < 0.05$ )

The results of Minimum Inhibitory Concentration (MIC) that had been obtained was

then incubated for 24 hours on *Sabouraud dextrose agar* (SDA) media and measurements of Minimum

Bactericidal Concentration (MBC) were carried out using colony counter to get the results as in Table

3.

**Table 3. Results of Colonies Number for Minimum Bactericidal Concentration (MBC) Determination of Mauli Banana Stem and Basil Leaves Extract Mixture Against *Candida albicans***

Group	N	Mean ± Standard Deviation
10%	3	1277.33 ± 68.039
20%	3	1157.33 ± 39.463
30%	3	984.00 ± 49.960
40%	3	531.00 ± 23.812
50%	3	424.00 ± 18.330
60%	3	389.33 ± 18.037
70%	3	360.00 ± 32.000
80%	3	291.00 ± 4.359
90%	3	257.33 ± 16.166
100%	3	153.33 ± 34.020
Negative control	3	1901.33 ± 28.095

Table 3 shows that mauli banana stem and basil leaves extract mixture at concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100% and negative control were still not capable to kill *Candida albicans* because the growth of *Candida albicans* was still observed on the *Sabouraud dextrose agar* (SDA) media.

The data analysis for MBC was later carried out using *Shapiro-Wilk* normality test and a value  $p > 0.05$  was obtained, which means that the data

were normally distributed. Then homogeneity test was performed using the *Levene's test* and a significance value  $p = 0.0183$  ( $p > 0.05$ ) was obtained, which means that the data is not homogeneous. *One Way ANOVA* analysis results obtained significance value  $p = 0.000$  ( $p < 0.05$ ), which means that there were significant differences of average between each treatment group. The data analysis was continued with the *Post Hoc Bonferroni test* in Table 4.

**Table 4. Post Hoc Bonferroni Test Results of Minimum Bactericidal Concentration (MBC) of Mauli Banana Stem and Basil Leaves Extract Mixture Against *Candida albicans***

Group	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%	Control (-)
10%		0.018*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
20%			0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
30%				0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
40%					0.055	0.003*	0.000*	0.000*	0.000*	0.000*	0.000*
50%						1.000	1.000	0.006*	0.000*	0.000*	0.000*
60%							1.000	0.116	0.006*	0.000*	0.000*
70%								1.000	0.080	0.000*	0.000*
80%									1.000	0.004*	0.000*
90%										0.072	0.000*
100%											0.000*
Control (-)											

Information :

\* = significant difference ( $p < 0.05$ )

## DISCUSSION

The mauli banana stem and basil leaves extract mixture at concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% have antifungal activity against *Candida albicans*. Mauli banana stem extract that is not mixed with basil leaves extract can only inhibit the growth of

*Candida albicans* at a concentration of 25% and basil leaves extract that is not mixed with mauli banana stem extract can only inhibit the growth of *Candida albicans* at a concentration of 12,5%.<sup>7,8</sup> Based on this, the mixing of basil leaves extract with mauli banana stem extract demonstrates a synergistic effect which increases antifungal

activity to inhibit the growth of *Candida albicans* with a minimum concentration of 10%.

The synergistic effect occurs when two or more antimicrobial substances are used together, thus causing a greater increase in the antimicrobial effect from one of the substances.<sup>10</sup> The enhancement of antifungal activity in inhibiting the growth of *Candida albicans* was caused by the synergistic effect from the tannin, saponin, and flavonoid contents in the banana Mauli stem and the content of eugenol, flavonoids, essential oils, tannins, and alkaloids in the basil leaves which both have antifungal activity.<sup>8,11</sup>

The tannin content in the Mauli banana stem and basil leaves operate together on the fungus cell wall. The tannin in the Mauli banana stem targets the cell wall polypeptide which causes damage to the fungus cell wall. Tannins in basil leaves are able to shrink the fungus cell walls resulting in disturbed cell activities and stunted growth.<sup>3,8</sup>

The hydroxyl group in the flavonoid compound from the Mauli banana stem causes changes in organic components and nutrient transport which results in toxic effect on the fungus. Flavonoids from basil leaves can inhibit fungal activity by lysing the cell walls that have been formed. Saponins in mauli banana stems are in the form of polar-shaped surfactants that are able to break down the fat layer in the fungus cell membrane which causes disruption of the cell membrane permeability and result in the diffusion process of materials or substances needed by the fungus to be disrupted.<sup>8,12</sup>

Alkaloids in basil leaves can damage the constituent components of peptidoglycan in cell walls which results in the incomplete formation of these components and will form holes or channels so that the cell membrane is leaked. Intracellular materials such as electrolytes (especially potassium compounds) are lost thus damaging and inducing the death of fungal cell. Eugenol from basil leaves can interact with fungus cells through an absorption

process that involves hydrogen bonds. At low levels, the phenol protein complex will be formed with weak bonds and immediately decompose. Furthermore, phenol will penetrate into the cell and cause protein denaturation. Essential oils in basil leaves disrupt the formation process of fungus cell membranes and fungus cell walls thus impeding its absolute formation.<sup>8</sup>

Concentration of mauli banana and basil leaves extract mixture of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% were still not capable to kill *Candida albicans* because the cell structure in *Candida Albicans* can form chlamyospore which thickens the cell wall and increases the difficulty to be penetrated by mauli banana stem and basil leaves extract mixture. The growth of *Candida albicans* on *Sabouraud dextrose agar* (SDA) media is suspected to be caused by insufficiency in the mother liquor concentration of 25% mauli banana stem extract and 12.5% basil leaves extract and limitation in initial concentration capacity to inhibit the growth of *Candida albicans* so that it is unable to kill *Candida albicans*.<sup>13</sup>

Based on this, further research can be tried to increase the concentration of the mother liquor to determine the Minimum Kill Concentration (MKC). This is in accordance with the research from Ornay et. al. (2017) which reveals that the killing ability of an antimicrobial substance was influenced by the concentration of the given substance. Increasing the concentration of mauli banana stem and basil leaves extract mixture results in a higher content of active ingredients that function as antifungal so that the ability to terminate *Candia albicans* growth will become greater.

## REFERENCES

1. Hu L, et al. Characterization of Oral Candidiasis and The *Candida* Species Profile in Patients with Oral Mucosal Diseases. *Microbial Pathogenesis*. 2019; 134 (103575): 1-5.
2. Darmawan W, Farida R, Redjeki S. The Effect of *Nigella Sativa* (Black Cumin) Seed Extract on *Candida albicans* Viability. *International Conference on Global Health*. 2019; 11(1): 88-91.
3. Permatasari, Budiarti LY, Apriasari ML. Efektivitas Antifungi Ekstrak Metanol Batang Pisang Mauli (*Musa Acuminata*) dan Chlorhexidine Gluconate 0,2% Terhadap *Candida albicans*. *Dentino (Jurnal Kedokteran Gigi)*. 2016; 1(1): 10-14.
4. Hatta I, Firdaus IWAK, Apriasari ML. The Prevalence of Oral Mucosa Disease of Gusti Hasan Aman Dental Hospital in Banjarmasin, South Kalimantan. *Dentino (Jurnal Kedokteran Gigi)*. 2018; 3(2): 211-214.

5. Dias IJ. Antifungal Activity of Linalool in Cases Of *Candida* Spp. Isolated from Individuals with *Oral* Candidiasis. *Brazilian Journal of Biology*. 2018; 78(2): 368-374.
6. Apriasari ML, Dachlan YP, Ernawati DS. *Potensi Bahan Alam Terhadap Penyembuhan Ulser Mukosa Mulut*. Jakarta: Salemba Medika; 2017. p.2-7.
7. Septianoor MH, Apriasari ML, Carabelly AN. Uji efektivitas antifungi ekstrak metanol batang pisang mauli (*Musa* sp) terhadap *Candida albicans*. *Jurnal PDGI*. 2013; 62(1): 7-10.
8. Ornay AKD, Prehananto H, Dewi ASS. Growth Inhibition of *Candida albicans* And Power Kill *Candida albicans* Extract Basil Leave. *Jurnal Wiyata*. 2017; 4(1): 78-83.
9. Saputri MV, Carabelly AN, Firdaus IWAK. Toxicity Test of the Mixed Mouthwash of Mauli Banana Stem and Basil Leaf Against Fibroblast Cell Study in Vitro. *Dentino (Jurnal Kedokteran Gigi)*. 2019; 4(2): 151-155.
10. Syamsudin. *Interaksi Obat: Konsep Dasar dan Klinis*. Jakarta: UI-Press; 2011. p.78-85.
11. Puspitasari D, Apriasari ML. Effect of 25%, 37,5% And 50% Mauli Banana (*Musa Acuminata*) Stem Extract as Mouthwash on Color Stability of Bulk Fill Resin Composite. *Dentino (Jurnal Kedokteran Gigi)*. 2018; 3(2): 168-173.
12. Apriasari ML. *Buku Referensi Potensi Batang Pisang Mauli (Musa acuminata) Sebagai Obat Topikal Pada Penyembuhan Luka Mulut*. Banjarmasin: PT. Grafika Wangi Kalimantan; 2015. p.3.
13. Zahrah H, Mustika A, Debora K. Aktivitas Antibakteri dan Perubahan Morfologi dari *Propionibacterium Acnes* Setelah Pemberian Ekstrak *Curcuma Xanthorrhiza* *Jurnal Biosains Pascasarjana*. 2018; 20 (3): 1-10.