# *The effects of mangrove stem extract compared to mangrove leaf extract in inhibiting Streptococcus mutans and Candida albicans growth on acrylic plate*

Perbandingan pengaruh ekstrak batang mangrove dengan ekstrak daun mangrove dalam menghambat pertumbuhan *Streptococcus mutans* dan *Candida albicans* pada plat akrilik

**[1]Andi Adytha Mutiah Itte Rusiaty, [2]Moh. Dharma Utama, [2]Muh. Ikbal, [2]Eri H. Jubhari**
[1]Prosthodontic Specialistic Program,
[2]Department of Prosthodontic
Faculty of Dentistry Hasanuddin University,
Makassar, Indonesia
E-mail: andiadythamutiah@gmail.com

**ABSTRACT**
**Objective**: This study aimed to determine the effects of mangrove (*Avicennia marina*) stem extract compared to the leaf extract in inhibiting the growth of *S. mutans* and *C. albicans* on acrylic plate. **Methods**: Mangrove stems and leaves extract was made with concentrations of 5%, 10%, 20% and cleaning agents as the control. The acrylic plates were soaked in the mangrove extracts for 10 minutes. The calculation of the number of bacterial and fungal colonies were performed afterward, then the data were analyzed by *one way Anova* and *post hoc test* (Tukey HSD). **Results:** The number of colonies of mangrove stems extract were calculated for *C. albicans* with concentrations of 5% = 558.6, 10% = 264.8, 20% = 212.2, and control = 4.4, whereas the *S.mutans* with concentrations of 5% = 161.4, 10% = 54.6, 20% = 37.2, and the control = 131. Statistical analysis of mangrove leaves extract with concentrations of 5%= 7.185, 10% = 8.360, 20% = 8.485, and the control 9.105 showed the different significant zones of inhibition of *S.mutans* based on the control. The 5% of concentrations of mangrove leaves extract showed the inhibition activities against *S.mutans* but did not show the inhibition zone against *C.albicans*, while the 5% concentrations of mangrove stem extract showed the inhibition activities against *S.mutans* and *C.albicans*. **Conclusions:** Mangrove stems extracts were more effective than the leaves extracts in inhibiting the growth of *S. mutans* and *C. albicans* on acrylic plate. The mangrove extracts, both the stems and leaves extracts, had higher effectiveness in inhibiting *S. mutans* compared to *C. albicans* activities.
**Keywords**: acrylic plate, *Candida albicans*, mangrove leaf extract (*Avicennia marina*), mangrove stem extract, *Streptococcus mutans*

**ABSTRAK**
**Tujuan**: Untuk mengetahui pengaruh ekstrak batang mangrove *(Avicennia marina)* dibandingkan dengan ekstrak daun dalam menghambat pertumbuhan *S.mutans* dan *C.albicans* pada plat akrilik. **Metode**: Ekstrak batang dan daun mangrove dibuat dengan konsentrasi 5%, 10%, 20% dan bahan pembersih sebagai kontrol. Plat akrilik direndam di dalam ekstrak mangrove selama 10 menit. Perhitungan jumlah koloni bakteri dan jamur dilakukan sesudahnya, kemudian data dianalisis dengan *one way* Anova dan *post hoc test* (Tukey HSD). **Hasil**: Jumlah koloni ekstrak batang mangrove dihitung untuk *C.albicans* dengan konsentrasi 5% = 558,6, 10% = 264,8, 20% = 212,2, dan kontrol = 4,4, sedangkan *S.mutans* dengan konsentrasi 5% = 161,4, 10% = 54,6, 20% = 37,2, dan kontrol = 131. Analisis statistik ekstrak daun mangrove dengan konsentrasi 5% = 7,185, 10% = 8,360, 20% = 8,485, dan kontrol 9,105 menunjukkan perbedaan signifikan zona penghambatan *S.mutans* berdasarkan kontrol. 5% konsentrasi ekstrak daun mangrove menunjukkan aktivitas penghambatan terhadap *S.mutans* tetapi tidak menunjukkan zona penghambatan terhadap *C.albicans*, sedangkan 5% konsentrasi ekstrak batang mangrove menunjukkan aktivitas penghambatan terhadap *S.mutans* dan *C.albicans*. **Simpulan**: Ekstrak batang mangrove lebih efektif daripada ekstrak daun dalam menghambat pertumbuhan *S.mutans* dan *C.albicans* pada plat akrilik. Ekstrak mangrove, baik ekstrak batang dan daun, lebih efektif menghambat *S.mutans* dibandingkan dengan aktivitas *C.albicans*.
**Kata kunci**: plat akrilik, *Candida albicans*, ekstrak daun mangrove *(Avicennia marina)*, ekstrak batang mangrove, *Streptococcus mutans*

## INTRODUCTION

Plaque accumulation can induce the growth of pathogenic microorganism on acrylic denture base and would cause an inflammation of the oral mucosa.[1-4] *Streptococcus mutans* and *Candida albicans* are some of the microorganisms that play the role in the inflammation, such as denture stomatitis.[5] Mangrove *(Avicennia marina)* is one of the natural materials

which contains of alkaloid, saponin, tannin, flavonoid, triterpenoid, and glicoside that can be used to inhibit the bacterial activity of microorganism.[6] The previous study had proven that the growth of *S.mutans* and *C.albicans* on denture base can be inhibited by extract of *Avicennia marina* leaf and stem. Many studies have found the evidence which support some facts that mangrove extracts are potential against microbial pathogens and possess antifungal, antiviral, antibacterial, antitumour and anticancer abilities.[7] Methanol and chloroform extracts of *Avicennia marina* exhibited promising antimicrobial activity.[8] Activity of antibacterial of leave and stem extracts of mangrove such as Klebsiella pneumonia, Pseudomonas aeruginosa, Vibrio parahaemolyticus, Staphylococcus aureus, Vibrio cholerahave against human pathogens have been studied, and antimicrobial activity of different mangrove plants has also been reported by many authors.[9]

Mangrove extract, both the stems and leaves have been shown to be effective in inhibiting the activity of *S.mutans* and *C.albicans* growth. This study aimed to determine the effects of mangrove *(Avicennia marina)* stem extract compared to the leaf extract in inhibiting the growth of *S.mutans* and *C.albicans* on acrylic plate.

## MATERIALS AND METHODS

The mangrove stems were inserted into a jar, added by 96% of ethanol, stirred, then closed by the cover jar with a piece of alumunium foil inside, stir, and let the maceration process last for 2x24 hours. The waste and filtrate were separated using filter paper. The filtering results were inserted into the rotary rotavapor to separate the stem extract and the ethanol. The mangrove leaves were dried, weighed, and were put into a jar, then be dampened with two liters of methanol and soaked for 3 days. It could be stirred occasionally. The jar was closed and kept in a room protected from direct sunlight and the extracting procedure is the same as the stem of the mangrove as mentioned previously.

Prepare the medium of nutrient broth (NB) and potato dextrose broth (PDB) as much as 9 mL in tubes. Inserted 0.1 mL *S.mutans* suspension into NB medium, followed by acrylic plate into the tube. The *C.albicans* suspension then be inserted into PDB medium and followed by acrylic plate into the tube. Both tubes were incubated for 24 hours at $37^0$C for the *S.mutans* and $25^0$C for the *C.albicans.* Extract of mangrove stem and leave were made in concentrations of 5%, 10%, 20% and cleaning agents as the control. The acrylic plates that have been soaked before were removed from the tube and inserted into mangrove extracts, and be soaked for 10 minutes. After that, the plates were removed from the extracts then be placed in the tube which contains of sterile aquadest. Half mililiters of the aquadest then inserted into the petri discs, followed by nutrient agar (NA) medium for *S.mutans* and potato dextrose agar (PDA) for the *C.albicans*, then be homogenized, incubated for 24 hours at $37^0$C for *S.mutans* and $25^0$C for *C.albicans*, then the colonies could be calculated. The calculation of the number of bacterial and fungal colonies were done afterward, then the data were analyzed by *one way Anova* and *post hoc test* (Tukey HSD).

## RESULT

The number of colonies of mangrove stems extract were calculated for *C. albicans* with concentrations of 5% = 558.6, 10% = 264.8, 20% = 212.2, and control = 4.4, whereas the *S. mutans* with concentrations of 5% = 161.4, 10% = 54.6, 20% = 37.2, and the control = 131. Statistical analysis of mangrove leaves extract with concentrations of 5%= 7.185, 10% = 8.360, 20% = 8.485, and the control 9.105 showed the different significant zones of inhibition of *S. mutans* based on the control. The 5% of concentrations of mangrove leaves extract showed the inhibition activities against *S.mutans* but did not show the inhibition zone against *C.albicans,* while the 5% concentrations of mangrove stem extract showed the inhibition activities against *S. mutans* and *C. albicans.*

The significant difference can be seen in the concentrations of 20% compared to 5% of stem extract ($p<0.05$) in inhibiting *S.mutans* growth based on the number of colonies. All the concentrations of stem extract have the significant differences in inhibiting *C.albicans* growth except the concentration of 10% compared to the 20%. The extract of leaves in all concentrations were significant compared to the control in inhibiting *C.albicans* growth based on the inhibition zone, while the leaves extract in inhibiting *S.mutans* were all significant except for concentration of 5% compared to 10% and the 20% compared to the positive control.

## DISCUSSION

Mangrove stem and leaves extracts could inhibit *C.albicans* and *S.mutans* from the concentration of 5% until 20%. Stems extract were more effective in inhibiting *S.mutans* growth compared to *C.albicans.* Based on *post hoc test* Tukey HSD, the most effective stem extract concentration in inhibiting *S.mutans* and *C.albicans* growth were 20%. Amirkaveei, in his study also explained that Avicennia marina extract were more effective as an antibacterial than antifungi.[10] Extract of 10% mangrove leaves extract contains of

**Table 1** The effect of *mangrove* leaf extract in inhibiting *Streptococcus mutans* growth

| Intervention(i) | Comparison (j) | *Mean Difference (i-j)* | *p-value* |
|---|---|---|---|
| Leaf Extract (5%) | Leaf Extract (10%) | -0.365 | 0.506 |
| | Leaf Extract (20%) | -1.340 | 0.003* |
| | Positive Control | -2.150 | 0.001* |
| Leaf Extract (10%) | Leaf Extract (5%) | -0.365 | 0.506 |
| | Leaf Extract (20%) | -1.730 | 0.006* |
| | Positive Control | -1.875 | 0.001* |
| Leaf Extract (20%) | Leaf Extract (5%) | -1.340 | 0.003* |
| | Leaf Extract (10%) | -1.730 | 0.006* |
| | Positive Control | -0.663 | 0.079 |

**Post Hoc Test: Tukey's HSD (High Significant Difference)-test: p<0.05: significant*

**Table 2** The effect of *mangrove* leaf extract in inhibiting *Candida albicans* growth

| Intervention(i) | Comparison (j) | *Mean difference (i-j)* | *p-value* |
|---|---|---|---|
| Leaf Extract (5%) | Leaf Extract (10%) | -0.347 | 1.00 |
| | Leaf Extract (20%) | -0.554 | 0.99 |
| | Positive Control | -0.627 | 0.001* |
| Leaf Extract (10%) | Leaf Extract (5%) | -0.347 | 1.00 |
| | Leaf Extract (20%) | -0.296 | 1.00 |
| | Positive Control | -6.473 | 0.002* |
| Leaf Extract (20%) | Leaf Extract (5%) | -0.554 | 0.99 |
| | Leaf Extract (10%) | -0.296 | 1.00 |
| | Positive Control | -6.035 | 0.003* |

**Post Hoc Test: Tukey's HSD (High Significant Difference)-test: p<0.05: significant*

**Table 3** The effect of *mangrove* stem extract in inhibiting *Candida albicans* growth

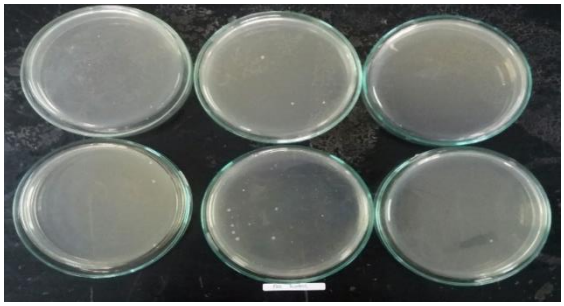
| Intervention(i) | Comparison (j) | *Mean Difference (i-j)* | *p-value* |
|---|---|---|---|
| Stem Extract (5%) | Stem Extract (10%) | 305.800* | .000 |
| | Stem Extract (20%) | 346.400* | .000 |
| | Positive Control | 554.200* | .000 |
| Stem Extract (10%) | Stem Extract (5%) | -305.800* | .000 |
| | Stem Extract (20%) | 40.600 | .901 |
| | Positive Control | 248.400* | .003 |
| Stem Extract (20%) | Stem Extract (5%) | -346.400* | .000 |
| | Stem Extract (10%) | -40.600 | .901 |
| | Positive Control | 207.800* | .014 |
| Positive Control | Stem Extract (5%) | -554.200* | .000 |
| | Stem Extract (10%) | -248.400* | .003 |
| | Stem Extract (20%) | -207.800* | .014 |

**The mean difference is significant at the 0.05 level*

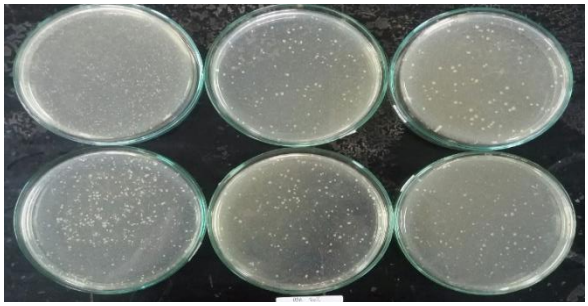
**Table 4** The effect of *mangrove* stem extract in inhibiting *Streptococcus mutans* growth

| Intervention(i) | Comparison (j) | *Mean Difference (i-j)* | *p-value* |
|---|---|---|---|
| Stem Extract (5%) | Stem Extract (10%) | 107.200 | .065 |
| | Stem Extract (20%) | 124.600* | .028 |
| | Positive Control | 30.800 | .862 |
| Stem Extract (10%) | Stem Extract (5%) | -107.200 | .065 |
| | Stem Extract (20%) | 17.400 | .970 |
| | Positive Control | 76.400 | .251 |
| Stem Extract (20%) | Stem Extract (5%) | -124.600* | .028 |
| | Stem Extract (10%) | -17.400 | .970 |
| | Positive Control | -93.800 | .121 |
| Positive Control | Stem Extract (5%) | -30.800 | .862 |
| | Stem Extract (10%) | 76.400 | .251 |
| | Stem Extract (20%) | 93.800 | .121 |

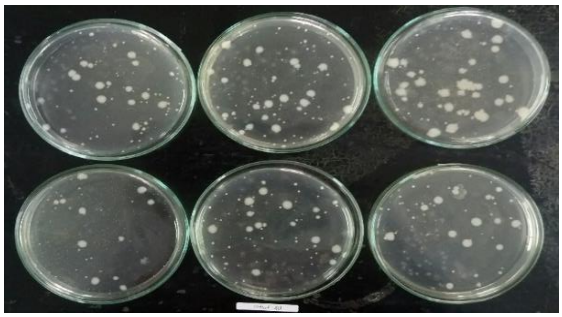
**The mean difference is significant at the 0.05 level.*



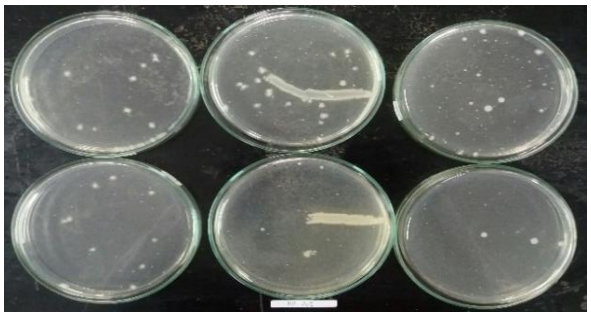
**Picture 1** Positive control for *C.albicans*



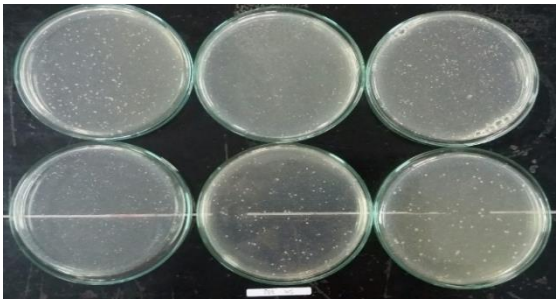
**Picture 2** *C.albicans* colonies in 20% of extracts



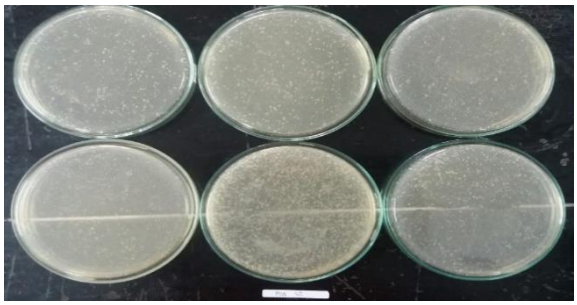
**Picture 3** *C.albicans* colonies in 10% of extracts



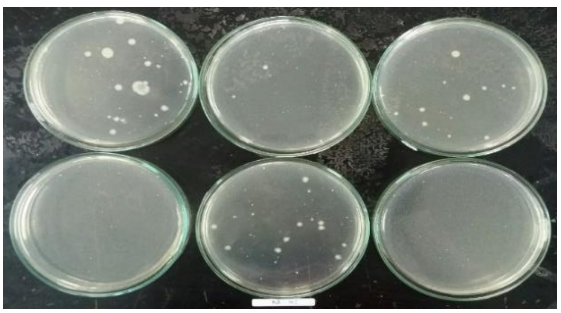
**Picture 4** *C.albicans* colonies in 5% of extracts



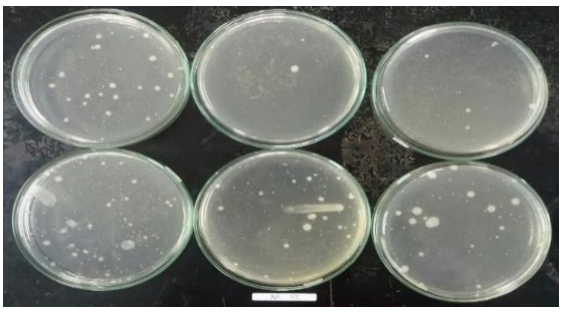
**Picture 5** Positive control for *S.mutans*



**Picture 6** *S.mutans* colonies in 20% of extracts



**Picture 7** *S.mutans* colonies in 10% of extracts



**Picture 8** *S.mutans* colonies in 5% of extracts

active material which has an effect in inhibiting the growth of *S.mutans*, as mentioned by Pelczar and Chan, the higher concentration the faster material will inhibit bacterial growth. Mangrove leaves extract at the concentration of 10% did not show an inhibition activity against *C.albicans* due to minimum flavonoid level as bacteriostatic agent. This statement was similar to the research conducted by Dharmautama, which explained that the mangrove leaves extract could not inhibit *C.albicans* in the concentration of 10% or lower.[11] Mangrove stems extracts were more effective than the leaves extracts in inhibiting the growth of *S.mutans* and *C.albicans* on acrylic denture base due to higher concentration needed for the leaves extract to show inhibition activity. The mangrove extract, both the stems and leaves extracts, had higher effectiveness in inhibiting *S.mutans* compared to *C. albicans* activities.

Based on this study, it was concluded that *Avicennia marina* extract can inhibit the activity of *C. albicans* and *S.mutans* in concentration of 5%, 10% and 20%. The mangrove extracts, both the stems and

leaves extracts, had higher effectiveness in inhibiting *S.mutans* compared to *C.albicans*. Mangrove stem extracts were more effective than the leaves extracts in inhibiting the growth of *S.mutans* and *C.albicans* on acrylic plate due to higher concentration needed for the leaves extract to show the inhibition activity.

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