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Research Report

Effect of kombucha butterfly pea (*Clitoria ternatea*) solution on *Enterococcus faecalis* biofilm

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ABSTRACT

Background: *Enterococcus faecalis is a facultative anaerobic bacterium that is often found in the root canals of teeth with pulp necrosis and is often the cause of endodontic treatment failure. On the other hand, the fermented beverage kombucha is made using a fermentation process by bacteria and yeast, known as SCOBY (Symbiotic Culture of Bacterial and Yeast). The fermentation process in kombucha causes a decrease in the pH of the medium, where the bacteria in the SCOBY convert sugar into ethanol and acetic acid. The butterfly pea flower (Clitoria ternatea) has single petals of purple, blue, pink, or white color and is proven to have antibacterial as well as antibiofilm properties.* ***Purpose:*** *To determine the effect of kombucha butterfly pea (Clitoria ternatea) solution on Enterococcus faecalis biofilm.* ***Methods:*** *Kombucha butterfly pea with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56%, as well as NaOCl 2,5% was given Enterococcus faecalis biofilm. To determine biofilm formation, test tubes were washed, adherent cells were stained with 0.1% crystal violet, and light absorbance was measured with a spectrophotometer at a wavelength of 540nm.* ***Results:*** *The percentage of inhibitory power of kombucha butterfly pea against E. faecalis biofilm decreased gradually from concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56%. The highest percentage of inhibition was in kombucha with butterfly pea with a concentration of 100% which is equivalent to 2,5% NaOCl as an antibiotic for root canal irigation.* ***Conclusion:*** *Kombucha of butterfly pea flower (Clitoria ternatea) can reduce the decrease in Enterococcus faecalis biofilm formation with a concentration of 12.5% designated as $MBIC_{50}$ and a concentration of 100% designated as $MBEC_{90}$.*

Keywords: *Butterfly Pea (Clitoria ternatea), Kombucha, Antibiofilm, Enterococcus faecalis*

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INTRODUCTION

The body's overall health is significantly influenced by oral health. According to Indonesia's 2018 Basic Health Research (Riskesdas) report, 45,3% of the country's population has dental caries[1]. Caries is a dental condition caused by the accumulation of plaque formed from food residues. If caries is not treated, it can cause infection of the tooth root canals and disruption of the pulp nerve.[1] Left untreated, caries can progress to pulp disease, which will then become periapical disease.[2]

Root canal treatment is an endodontic treatment performed by removing all necrotizing pulp tissue and forming a root canal to prevent recurrent infection. The causes of root canal failure can be said to be due to incomplete cleaning and formation of root canals, causing a lack of ability to eliminate existing microorganisms.[3]

Endodontics treatment failures are linked to persistently presence of bacteria, microleakage at the crown, inadequately cleaned and filled root canals, as well as missed canals. *Enterococcus faecalis* can survive in dentine tubules within the system, endure challenging conditions like starvation and an alkaline pH, and form biofilms, contributing to its continued existence despite receiving root canal therapy.[4]

Enterococcus faecalis is a type of facultative anaerobic bacteria that is often found in the root canals and is often the cause of failure in endodontic treatment.[5] *Enterococcus faecalis* was found nine times more often in post-root canal treatment infections than in primary infections. *Enterococcus faecalis* can form biofilms on various surfaces, both biological and abiotic.[6]

Collections of planktonic cells known as biofilms are enclosed in a matrix of Extracellular Polymeric Substances

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(EPS) that the microorganisms manufacture and are permanently adhered to a specific surface. This makes biofilms more complex and often more resistant to external factors compared to microorganisms in planktonic form.[7]

The irrigation materials often used to clean root canals are *Sodium hypochlorite* (NaOCl) 2.5%, and *EthylenediamineTetra-Acetic acid* (EDTA) 17%. The combination of NaOCl 2.5% and EDTA 17% is an effective combination of materials in root canal irrigation. NaOCl 2.5% is most often used in root canal treatment because it can remove organic components, and functions as an antimicrobial but is less effective in removing inorganic components, while EDTA can remove inorganic components, but its shortcomings are less effective in removing organic components.[1]

The natural material studied was kombucha butterfly pea (*Clitoria ternatea*). A group of bacteria and yeast known as SCOBY (Symbiotic Colony or Culture of Bacteria and Yeast) are responsible for the sour fragrance of kombucha, a fermented beverage.[8] Bacteria in SCOBY play a role in the fermentation process, including *Saccharomyces cerevisiae,* which converts sugar into ethanol and further into acetic acid by *Acetobacter xylinum.*[9]

Kombucha contains one of the metabolite compounds that have the potential to prevent bacteria, which is acetic acid. Acetic acid that has been formed during the fermentation process of kombucha butterfly pea ideally has the potential to reduce pH from acidic conditions to very acidic. pH derived from substrate concentration has the ability to affect the growth of pathogenic bacteria.[10]

Furthermore, the butterfly pea, also known as the telang flower (*Clitoria ternatea)*, is a unique flower with single petas that are purple, blue, pink, and white. The color of telang flowers indicates anthocyanin compounds that act as antioxidants. Anthocyanins when accompanied by kombucha fermentation, will make this compound more stable when fermented with LAB (Lactic Acid Bacteria), so at temperature, pH, and PPO (Polyphenol Oxidation) enzymes. The combination of these compounds provides potential antibiofilm activity against *Enterococcus faecalis* biofilms, with acetic acid playing a role in lowering the pH of the environment and antibiofilm compounds inhibiting biofilm formation or growth.[10,11]

Research conducted by Pertiwi F. showed that kombucha butterfly pea with a concentration of 40% white granulated sugar was the best concentration in inhibiting bacteria.[12] The purpose of this study is to evaluate the antibiofilm properties of kombucha butterfly (*Clitoria ternatea*) as an antibiofilm solution on *Enterococcus faecalis* biofilm as seen by the decline in in the development of *Enterococcus faecalis* biofilms. The biofilm's Optical Density (OD) value following treatment was used to measure the reduction in *Enterococcus faecalis* biofilm development.[13]

This study used serial dilutions of antibiofilm solutions added with *Enterococcus faecalis* biofilm. Furthermore, the effectiveness of kombucha butterfly pea antibiofilm against *Enterococcus faecalis* biofilm was analyzed by calculating the Minimum Biofilm Inhibitor Concentration ($MBIC_{50}$) and Minimum Biofilm Eradication Concentration ($MBEC_{90}$).[12]

MATERIALS AND METHODS

This study is in vitro laboratory experimental research. The research design used was post-test only control group. The research sample used was *Enterococcus faecalis* bacterial stock obtained from the Research Center of the Faculty of Dentistry, Airlangga University.

Butterfly pea flowers obtained from the Wage area, Taman, Sidoarjo, were taken in as much as 500 grams in fresh condition, then cleaned in running water until clean to remove dirt that was still attached, and then dried. The dried flowers were stored in a clean container to be boiled and fermented by SCOBY.

Enterococcus faecalis bacterial culture to be used was taken from *Enterococcus faecalis* stock using a sterile osse and planted on a well. Then the microplate was incubated for 24 hours at 37°C. The culture was matched with the McFarland standard, then thinned to reach the McFarland standard of 0.5 or equivalent to $1.5x10^8$ CFU/ml.

The butterfly pea kombucha was prepared by drying butterfly pea flowers at 50°C for four hours. Then put dried butterfly pea flowers in tea bags of 12 grams each. Brewed it with 1000 ml of water at 100°C and added white granulated sugar at 40% of the amount of water for 10 minutes. The brew was stored in sterilized glass jars. Cooling the butterfly pea kombucha for 2 hours until it reaches room temperature. The addition of starter and SCOBY into the glass jar after the kombucha butterfly pea reaches room temperature. Covered with a cloth and store in a place not exposed to direct sunlight for 12- 14 days. Then kombucha butterfly pea was taken as much as 2 ml and diluted into several concentration levels; there are 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56%.

After anaerobic incubation, rinsing with sterile water, and staining was performed. The amount of biofilm was determined by measuring the biofilms that had been stained optical density (OD) using 540 nm light wave spectrophotometer.

RESULTS

The objective of this study was to analyze the effect of kombucha butterfly pea solution on *Enterococcus faecalis* biofilm, where the concentrations of kombucha butterfly pea used in this study are 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56%. The capacity of kombucha to inhibit *Enterococcus faecalis* biofilm development was assessed using this concentration. The effectiveness of kombucha butterfly pea against *Enterococcus faecalis* biofilm was examined and measured with a spectrophotometer set at wavelength of 540 nm, which is expressed in OD units. The results of the OD reading of the study can be seen in (Table 1).

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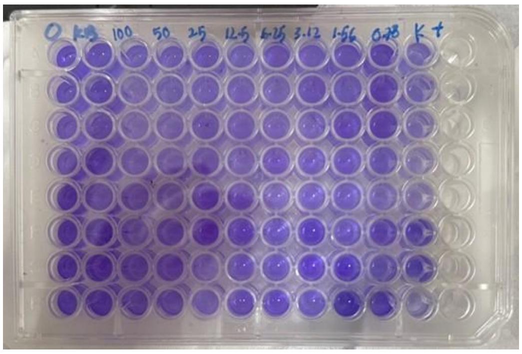

Figure 1. Microplate results after treatment.

Based on the results of reading, the OD of *E. faecalis* biofilm using a spectrophotometer, it was found that all treatments and the positive control had OD values that were lower than the OD value of the negative control. Figure 1 shows test bacteria soaked in each treatment group along with the positive formation control for 24 hours were stained with 200 µl of 0.1% CV dye for 15 minutes at room temperature. Distilled water was used to wash each biofilm, and adhering dye was extracted using 100 µl of 98% ethanol from the cells. The plates were shaken for five minutes.

Based on the biofilm OD readings presented in the table, calculations can be performed. Additionally, the average

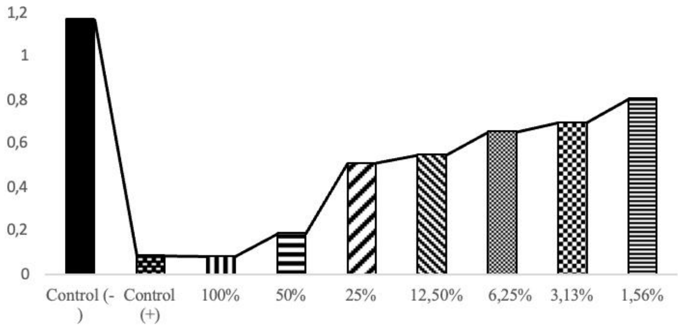

Figure 2. *E. faecalis* bacterial biofilm formation.

Table 1. *E. faecalis* bacterial biofilm

Sample	K(+)	K(-)	100%	50%	25%	12.5%	6.25%	3.125%	1.56%
1	0.064	0.816	0.095	0.284	0.581	0.52	0.719	0.911	0.758
2	0.061	1.687	0.108	0.224	0.577	0.604	0.662	0.792	0.611
3	0.106	1.603	0.079	0.117	0.421	0.673	0.696	0.712	0.881
4	0.052	0.986	0.091	0.113	0.441	0.438	0.643	0.745	0.839
5	0.075	0.903	0.055	0.358	0.467	0.542	0.657	0.705	0.845
6	0.149	1.257	0.087	0.121	0.582	0.543	0.58	0.677	0.720
7	0.083	1.074	0.069	0.16	0.505	0.441	0.676	0.984	0.962
8	0.096	1.011	0.099	0.125	0.505	0.617	0.594	0.926	0.816

Table 2. The mean concentration value and standard deviation of *E. faecalis* bacterial biofilm formation

Group	N	Mean Concentration ± Standard Deviation
Control (-)	8	1.167 ± 0.322
Control (+)	8	0.086 ± 0.031
100% Concentrated Kombucha Butterfly Pea	8	0.085 ± 0.017
50% Concentrated Kombucha Butterfly Pea	8	0.188 ± 0.092
25% Concentrated Kombucha Butterfly Pea	8	0.51 ± 0.065
12.5% Concentrated Kombucha Butterfly Pea	8	0.547 ± 0.083
6.25% Concentrated Kombucha Butterfly Pea	8	0.653 ± 0.047
3.125% Concentrated Kombucha Butterfly Pea	8	0.694 ± 0.059
1.56% Concentrated Kombucha Butterfly Pea	8	0.804 ± 0.107

Table 3. Mann Whitney Advanced Test Result

Group	100%	50%	25%	12,5%	6,25%	3,125%	1,56%	K(+)
100%	-	-	-	-	-	-	-	-
50%	0.000*	-	-	-	-	-	-	-
25%	0.000*	0.000*	-	-	-	-	-	-
12.5%	0.000*	0.000*	0.328	-	-	-	-	-
6.25%	0.000*	0.000*	0.001*	0.015*	-	-	-	-
3.125%	0.000*	0.000*	0.000*	0.001*	0.161*	-	-	-
1.56%	0.000*	0.000*	0.000*	0.001*	0.005*	0.021*	-	-
K(+)	0.721	0.002*	0.000*	0.001*	0.000*	0.000*	0.000*	-
K(-)	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.003*	0.000*

*Significant difference (p-value <0,05)

Table 4. pH Value of Kombucha Butterfly Pea

Group	pH
100% Concentrated Kombucha Butterfly Pea	2.12
50% Concentrated Kombucha Butterfly Pea	2.13
25% Concentrated Kombucha Butterfly Pea	2.17
12.5% Concentrated Kombucha Butterfly Pea	2.37
6.25% Concentrated Kombucha Butterfly Pea	2.52
3.125% Concentrated Kombucha Butterfly Pea	2.72
1.56% Concentrated Kombucha Butterfly Pea	3.23

OD value of *E. faecalis* biofilm for each group can be determined (Table 2).

A schematic comparing the positive, negative, and treatment control groups is presented in Figure 2. According to the study's findings, the biofilm's average value and standard deviation were as follows: The negative control group's biofilm formation value was 1.167 ± 0.322, then the biofilm value decreased in the positive control group by 0.086 ± 0.031, the 100% concentration group was 0.085 ± 0.017, the 50% concentration group was 0.188 ± 0.092, the 25% concentration group was 0.51 ± 0.065, the 12.5% concentration group was 0.547 ± 0.083, the 6.25% concentration group was 0.653 ± 0.047, the 3.125% concentration group was 0.694 ± 0.059, and the 1.56% concentration group was 0.804 ± 0.107.

When compared to the positive group, the negative control group's concentration dropped, the concentration groups of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56% were respectively 93%, 93%, 84%, 56%, 53%, 44%, 40%, and 31%.

The data were analyzed using statistical methods. Normality and homogeneity tests were performed prior to statistical analysis for each research group. The test was conducted using the Shapiro-Wilk Test to determine if the data was normally distributed, while the homogeneity test was conducted using the Levene Test to ensure the data was homogeneous.

Statistical analysis began with a normality test on each group using the Shapiro-Wilk Test ($n<50$) to determine whether the data had a normal distribution. The hypotheses used were: 1) H0 = regulary distributed data and 2) H1 = non-normally distributed data were the hypotheses that were employed.

The results of the Shapiro-Wilk Test showed a significance value (p-value) of 0.000 was obtained for the OD value of biofilm in the positive control, negative control, and all treatment groups. The p value which is smaller than α (0.05) indicates that H0 is accepted, so it can be concluded that the research data is normally distributed.

Following the normality test, each group underwent a homogeneity test using the Levene test to ascertain whether the data was homogenous. H0 = homogeneous data and H1= non-homogeneous data, were the hypotheses that were employed.

The Levene Test findings where the mean OD of biofilm in the positive control, negative control, and all treatment groups showed a significant value (p-value) of 0.000. H1 is approved since the p-value for each category is less than α (0.05), indicating that the study data is not homogeneous.

Based on these results, the data were analyzed using a non-parametric test, the Kruskal-Wallis test. From the results of the test, a p-value of 0.000 ($P<0.05$) was obtained, which indicated a significant effect of all kombucha butterfly pea treatment groups on the formation of *Enterococcus faecalis* biofilm.

Therefore, further testing was carried out to determine which groups had differences. The hypotheses used were: H0 = there was no difference between the groups, while H1 = there was a difference between groups. Additional testing was done in this study using the Mann Whitney Test to identify significant differences between treatment groups.

A significant value (p-value) of 0.000 is displayed in Table 3 for every comparison between the negative control group, the positive control group, and almost all kombucha butterfly pea treatment groups except for the 100% concentration group and positive control and concentrations of 25% and 12.5%. When comparing the positive control group, negative control group, and nearly all treatment groups, the p-value is less than the α value (0.05), while the 100% concentration group and positive control and concentrations of 25% and 12.5% have p-values greater than the α value (0.05).

This shows that H0 is rejected, so it can be concluded that there is a significant difference between the negative control group and the positive control group and almost all treatment groups except for the 100% concentration group and positive control and concentrations of 25% and 12.5%.

From the Mann Whitney Test, it is evident that nearly all treatment groups exhibit notable variations in their ability to suppress the production of biofilms (indicated with an asterisk).

Table 4 shows the pH values in all treatment groups of kombucha butterfly pea. The pH scale is used to determine how acidic or alkaline a solution is. The pH scale has values between 0 and 14, with a value of 7 as the neutral point. A pH value below 7 indicates an acidic solution, while a pH value above 7 indicates a basic solution.[14] The results of pH value measurements were obtained, where all groups had values below 7 and could be said to be acidic solutions.

DISCUSSION

The findings of this study demonstrated that there was an effect of reducing biofilm formation by kombucha butterfly pea solution on *Enterococcus faecalis*. *Enterococcus faecalis* is a type of facultative anaerobic bacteria that is often found in root canals of teeth that experience pulp necrosis and is often the cause of failure in endodontic treatment.[6] Previous studies have searched certain ways to eliminate *Enterococcus faecalis*.[15-17] *Enterococcus faecalis* was found nine times more in post-endodontic infections than in primary infections.[9]

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The kombucha butterfly pea with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and positive control had much smaller Optical Density (OD) values and a higher percentage of inhibition compared to the untreated biofilm group or negative control. This indicates that kombucha butterfly pea with concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and positive control has a significant ability to inhibit the growth of *Enterococcus faecalis* biofilm.

A 100% concentration of kombucha butterfly pea has the highest inhibitory power of 93% compared to other concentrations. Yuanita *et al*. claimed that as concentration increases, the greater the antibiofilm strength contained in it, and the lower the OD value of the biofilm formed by *Enterococcus faecalis* bacteria.[4]

Additionally, the sample that can inhibit at least 50% of biofilm formation – known as the Minimal Biofilm Inhibition Concentration, or $MBIC_{50}$ – is determined using the percentage data collected.[7] According to the results, kombucha butterfly pea with a concentration of 12.5% produced an inhibition power of 53%, which indicates that the $MBIC_{50}$ value is at a concentration of 12.5%.

After the $MBIC_{50}$ was known in this study, the next data was used to calculate the Minimum Biofilm Eradication Concentration $MBIC_{90}$ value, which is the lowest concentration of a solution needed to eliminate at least 90% of the total biofilm formed.[9] Based on the results of the study, butterfly pea flower kombucha with a concentration of 100% can reduce biofilm formation by 93%, which indicates that the $MBEC_{90}$ value is at a concentration of 100%.

From the results of pH measurements on kombucha butterfly pea with various concentrations, it can be seen that higher concentrations result in lower pH values, whereas lower concentrations result in higher pH values. tends to increase. At a concentration of 100%, the pH value of kombucha is 2.12, which indicates a fairly high level of acidity. Meanwhile, at the lowest concentration of 1.56%, the pH increased to 3.23, but remained in the acidic range because it was below pH 7. At the pH of the seven treatment groups, Enterococcus faecalis bacteria could not grow optimally because the ideal environmental pH for its growth ranges from 4 to 11, and changes in pH in these bacterial cells will inhibit the process of sending amino acid RNA, thus inhibiting bacterial growth.[18]

The fermentation process of kombucha butterfly pea involves interactions between microorganisms in the SCOBY and active compounds from butterfly pea flowers. During fermentation, microorganisms in the SCOBY convert sugar into organic acids such as lactic acid and acetic acid, which can lower the pH and create an acidic environment. This condition can affect the stability of *Enterococcus faecalis* biofilm.[11] In addition, butterfly pea flowers are known not to contain lactic acid and acetic acid but have the ability to act as an antibiofilm against *Enterococcus faecalis* when secondary metabolite substances such terpenoids, alkaloids, flavonoids, tannins, and saponins are present.[12,19,20]

In conclusion, each group of these compounds has the ability to reduce biofilm formation through various mechanisms. For example, alkaloids and terpenoids can cause leakage of intracellular components, which leads to cell death. Tannins and flavonoids reduce the ability of bacteria to adhere and interfere with protein synthesis for cell wall formation. Saponins can cause leakage of important components, which results in lysis or cell death.[10] The anthocyanin content as an antioxidant in butterfly pea flowers can help regenerate damaged tissue and reduce inflammation of tooth root tissue due to bacterial infection. Thus, the fermentation results of both make the butterfly pea kombucha an antibiofilm solution that combines the probiotic benefits of kombucha and the bioactive activity of butterfly pea flowers, which provide a stronger and more effective effect in reducing biofilm formation compared to each component used separately.

REFERENCES

1. Wicaksono DA, Suling PL, Mumu JY. Efektivitas ekstrak daun mangrove *Bruguiera gymnorrhiza* terhadap bakteri *Enterococcus faecalis* sebagai alternatif larutan irigasi perawatan saluran akar. e-GiGi. 2024;13(1):7–14.
2. Rumate DE, Wicaksono DA, Yuliana Y. Kepatuhan pasien menjalani perawatan saluran akar multi kunjungan di Rumah Sakit Gigi dan Mulut Universitas Sam Ratulangi. e-GiGi. 2023;11(2):176–82.
3. Ningsih JR, Pradana FAJ. Karakteristik bakteri saluran akar pada gigi yang mengalami kegagalan perawatan saluran akar. In: Prosiding Dental Seminar Universitas Muhammadiyah Surakarta. 2023. p. 84–96.
4. Yuanita T, Setyabudi, Wahjuningrum DA, Wijayanto O, Chang NA, Juniarti DE, Wahjudianto N, Pawar AM, Bhardwaj A, Prasetyo EP. Antibiofilm activity of epsilon polylysine (e-PL) against *Enterococcus faecalis* biofilm as a root canal dressing candidate. J Int Dent Med Res. 2024;17(4):1818-23.
5. Prasetya RC, Fatimatuzzahro N, Ermawati T, Kristina S, Prabaningrum RRH. Antibacterial Activity of Robusta Coffee (*Coffea Canephora*) Husk Extract Against *Enterococcus faecalis* and *Phorphyromonas gingivalis*: In Vitro Study. Trends Sci.2024;21(3):7303.
6. Koo H, Allan RN, Howlin RP, Stoodley P, Hall-Stoodley L. Targeting microbial biofilms: current and prospective therapeutic strategies. Nat Rev Microbiol. 2017;15(12): 740–55.
7. Hamzah H, Hertiani T, Pratiwi SUT, Nuryastuti T. Efek saponin terhadap penghambatan planktonik dan mono-spesies biofilm *Candida albicans* ATCC 10231 pada fase pertengahan, pematangan dan degradasi. Maj Farmaseutik. 2021;17(2):198–205.
8. Sinaga P, Marcellina S, Aritonang D. Teh kombucha bunga telang sebagai pilihan bisnis wirausaha berkelanjutan. J-CoSE J Community Serv Empower. 2024;2(1):16–25.
9. Irdawati I, Sari PA. Kombucha tea production by amobil cells in several different tea processing. Biosci. 2020;4(2): 133–139.
10. Abdilah NA, Rezaldi F, Kusumiyati K, Sasmita H, Somantri UW. Aktivitas antibakteri kombucha bunga telang (*Clitoria ternatea* L.) yang difermentasi dengan gula aren pada konsentrasi berbeda. Tirtayasa Med J. 2022;1(2):29–39.
11. Rezaldi F, Nurmaulawati R, Susilowati AA, Waskita KN, Puspita S, Rosalina V. Antimikroba pada produk bioteknologi

DOI: https://doi.org/10.20473/cdj.v15i2.2025.62-67

farmasi berupa sediaan obat kumur kombucha bunga telang (*Clitoria ternatea* L). J Ilm Farm Attamru (JIFA). 2022;3(2):1–16.

12. Pertiwi F. Antibakteri *Clostridium botulinum* dari bunga telang (*Clitoria ternatea* L) melalui metode bioteknologi fermentasi kombucha. Tirtayasa Med J. 2022;2(1):1–8.
13. Lynch MJ, Martin KA. Assessment of bacterial growth via optical density. J Microbiol Methods. 2020;172:172.
14. Unawahi S, Widyasanti A, Rahimah S. Ekstraksi antosianin bunga telang (*Clitoria ternatea* Linn) dengan metode ultrasonik menggunakan pelarut aquades dan asam asetat. J Trop Agric Eng Biosyst. 2022;10(1):1–9.
15. Prasetyo EP, Juniarti DE, Sampoerno G, Wahjuningrum DA, Budi AT, Hasri D, Tjendronegoro E. The antibacterial efficacy of calcium hydroxide-iodophors and calcium hydroxide-barium sulfate root canal dressings on Enterococcus faecalis and Porphyromonas gingivalis in vitro. Dent J. 2022;55(2):62-6.
16. Ismiyatin K, Cahyani F, Soetojo A, Widjiastuti I, Pribadi N, Nurkhalidah BN, Raftiani AS, Pramesty AK, Anindya C. Epigallocatechin-3-gallate (EGCG) and tricalcium silicate (C_3S) combination as an antibacterial agent against Enterococcus faecalis. Conservative Dentistry Journal. 2025;15(1):46-8.
17. Wahjuningrum DA, Setyabudi, Yuanita T, Wijayanto O, Chang NA, Juniarti DE, Wahjudianto N, Pawar AM, Bhardwaj A, Prasetyo EP. Antibiofilm activity of epsilon polylysine (e-PL) against Enterococcus faecalis biofilm as a root canal dressing candidate. J Int Dent Med Res. 2024;17(4):1818-23.
18. Syam S, Arifin NF, Anas R. Perbedaan daya hambat ekstrak daun jambu biji (*Psidium guajava* Linn.) dengan air perasan jeruk nipis (*Citrus aurantifolia*) sebagai bahan irigasi saluran akar penghambat bakteri *Enterococcus faecalis*. Makassar Dent J. 2019;8(1).
19. Yuanita T, Firmansyah AB, Ulfadi BTP, Prasetyo EP, Wahjuningrum DA. The effect of butterfly pea flower (Clitoria ternatea L.) kombucha against Streptococcus viridans. Conservative Dentistry Journal. 2025;15(1): 14-7.
20. Handriutomo YK, Soesilo D, Aprilia A, Parishni K, Rayhan R, Cahyani F. Effectiveness of Nipah leaf extract (Nypa fruticans) against Streptococcus mutans biofilm as cavity cleanser. Conservative Dentistry Journal. 2025;15(1): 23-6.