

Antibacterial Activity Test of Ethyl Asetate Fraction of Gletang Growth (*Tridax procumbens L.*) on The Growth of *Enterococcus faecalis*

Widyawati¹ , Ulima Audia Fayi Arta², Okmes Fadriyanti¹ 

¹ Department of Conservative, Faculty of Dentistry, Baiturrahmah University, Padang, Indonesia

² Student of the Faculty of Dentistry, Baiturrahmah University, Padang, Indonesia

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ABSTRACT

Background: Intractable root canal infection remains a serious obstacle in endodontic therapy and is a frequent cause of treatment failure, with *Enterococcus faecalis* (*E. faecalis*) as a key pathogen. This Gram-positive bacterium can persist under harsh conditions within the root canal system. Chlorhexidine is commonly used as a chemical irrigant, but its long-term application is associated with undesirable side effects, prompting the search for natural antibacterial alternatives. One promising candidate is the gletang plant (*Tridax procumbens L.*), which contains bioactive compounds with potential antimicrobial effects. **Objective:** This study aimed to evaluate the antibacterial activity of the ethyl acetate fraction of *Tridax procumbens L.* at various concentrations against *E. faecalis*. **Method:** A laboratory experimental design with post test only control group was used. Antibacterial testing was performed using the agar diffusion method with the gletang ethyl acetate fraction at 2%, 4%, 6%, and 8%, chlorhexidine as a positive control, and 96% methanol as a negative control. Data were analyzed univariately in tables and bivariately using Kruskal–Wallis and Mann–Whitney tests. **Results:** The fraction inhibited *E. faecalis* growth, with the largest mean inhibition zone of 3.6 mm at 8% and the smallest at 2%, 1.6 mm. Overall activity across all concentrations was classified as weak; however, statistical analysis ($p=0.003$, $p<0.05$) confirmed a significant inhibitory effect. **Conclusion:** The ethyl acetate fraction of the gletang plant (*Tridax procumbens L.*) at 8% is effective as an antibacterial against *Enterococcus faecalis*, with an average inhibition zone diameter close to that of the positive control. The higher the concentration of the ethyl acetate fraction, the higher the bacterial inhibition.

Keywords: *Enterococcus faecalis*, inhibition zone, *Tridax procumbens L.*

Correspondence: Ulima Audia Fayi Arta, Student of the Faculty of Dentistry, Baiturrahmah University, Jl. Raya By Pass Km 15, Aie Pacah, Koto Tengah, Padang City, West Sumatra, 25176, Indonesia. Email: widyawati@fkg.unbrah.ac.id

INTRODUCTION

Root canal treatment is a treatment for pulp disease that involves removing the necrotic pulp from the root canal and replacing it with a filling material. It aims to keep the damaged tooth functioning properly and does not need to be extracted. Root canal treatment is carried out with three main stages: biomechanical preparation, sterilization, and hermetic root canal filling or obturation, also known as the endodontic triad.¹ PSA can fail, one of the main causes is microorganisms that remain after treatment or reappear after PSA. Microorganism Root canal treatment is a treatment for pulp disease that involves removing the necrotic pulp from the root canal and replacing it with a filling material. It aims to keep the damaged tooth functioning properly and does not need to be extracted. Root canal treatment is carried out with three main stages: biomechanical preparation, sterilization, and hermetic root canal filling or obturation, also known as the endodontic triad.¹ PSA can fail, one of the main causes is microorganisms that remain after treatment or reappear after PSA. Microorganisms are considered the main cause of persistent pulp-periapical abnormalities and play an important role in the infectious course of pulp and periapical diseases. Microorganisms commonly found in infected root canals include *Enterococcus faecalis*, *Streptococcus anginosus*, *Bacteroides Gracilis* and *Fusobacterium nucleatum*. However, the bacteria that are known to be the most resistant and most commonly found are *Enterococcus faecalis* bacteria.^{2,17} *Enterococcus faecalis* bacteria are found nine times more in infections after root canal treatment because it has pathogenic properties associated with oral infections and can cause root canal infections, marginal periodontitis, and abscesses. *Enterococcus faecalis* can survive in the root canal by passing through the dentinal tubules, forming a smear layer, and binding to the dentinal plug at the apex of the tooth.^{3,17}

Enterococcus faecalis bacteria have the ability to avoid instruments and irrigation materials used during biomechanical preparation, as well as being able to catabolize energy sources and can survive in various environments.^{4,17}

Irrigation solutions commonly used in root canal treatment include *chlorhexidine*, sodium hypochlorite, and ethylene diamine tetraacetic acid. *Chlorhexidine* is a broad-spectrum antibacterial solution that can be used as an irrigation solution because it has electrostatic properties on the surface of bacteria, although it must be combined again with other irrigation materials to be effective. Some studies show that 2% *chlorhexidine* is recommended as a final rinse because of its outward spectrum of antimicrobial activity, substance and ability to inhibit collagen degradation in addition, *chlorhexidine* does not irritate periapical tissues, is less toxic than other solutions and does not smell strong however, this ability depends on pH and the presence of organic components used while sodium hypochlorite also has antibacterial activity but, when using high concentrations will cause toxicity because it can damage periapical tissues and irritate tissues and smell bad.^{5,15,16} Irrigation solutions should have no toxic properties and minimal tissue damage to reduce treatment failure. Therefore, irrigation solutions may be replaced with more biocompetent materials, such as natural materials.

One of the plants that has natural properties is gletang (*Tridax procumbens L.*). Gletang (*Tridax procumbens L.*) is easily found as a wild weed, this plant can be used as a traditional medicine in either fresh form, or dried.^{6,16} The whole gletang plant (*Tridax procumbens L.*) has antimicrobial activity against various bacterial species. In addition, every part of this gletang plant is useful because it has pharmacological activities such as hepatoprotective effects, immunomodulating properties, antidiabetes, antimicrobial, anti-inflammatory, antioxidant, diarrhea, and dysentery. This gletang plant is rich in chemical

contents such as flavonoids, saponins, tannins, and terpenoids as major secondary metabolites.^{7,8,16}

MATERIALS AND METHODS

This research is a laboratory experimental study aimed to determine the antibacterial activity of the ethyl acetate fraction of gletang plant (*Tridax procumbens L.*) against the growth of *Enterococcus faecalis* bacteria. This study used all parts of gletang plants, including leaves, stems, flowers, and roots, obtained from Jalan By Pass, Padang city, West Sumatra. The processing of gletang plants begins with wet sorting to separate from dirt and other plants that are not needed, washing using clean and running water, drying by drying samples at room temperature without exposure to sunlight, and placed on a white cloth that absorbs water for 14 days. After that, the dried gletang plant samples were cut into small pieces and mashed using a blender to obtain 2 kg and then extracted.

The extraction process chosen is the maceration method. Maceration is a cold extraction method used for simplisia that has a soft texture. Methanol was chosen as the solvent in the maceration process because it is universal and can attract all types of active substances, both polar and semi-polar, so that the active compounds will be dissolved in it and the toxicity levels are relatively low.⁹ Extraction was carried out by immersing the sample in an Erlenmeyer tube until it was completely submerged, then stirring every 3x24 hours until a clear colored maserat was obtained. The mixture was evaporated using a vacuum rotary evaporator, and a thick extract weighing 41.2247 kg was obtained. The results of the thick extract obtained were carried out by the separation process using the column chromatography fractionation method. This research was ethical approval from the Ethics Committee of Baiturrahmah University with

registration number: 010/KEPK-FKGUNBRAH/18/12/2024.

RESULTS

The results of measuring the average diameter of the inhibition zone of the ethyl acetate fraction of gletang plant (*Tridax procumbens L.*) against the growth of *Enterococcus faecalis* bacteria can be seen in the following table.

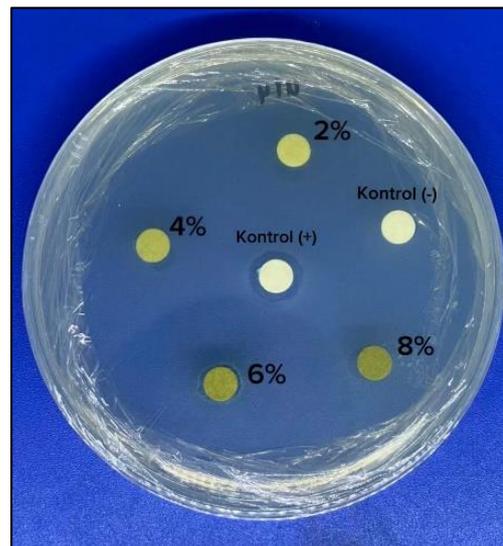


Figure 1. Antibacterial activity test results of the ethyl acetate fraction of the gletang plant (*Tridax procumbens L.*) at concentrations of 2%, 4%, 6%, and 8% against the growth of *Enterococcus faecalis* bacteria.

Table 1. Mean antibacterial activity of ethyl acetate fraction of gletang plant (*Tridax procumbens L.*) against the growth of *Enterococcus faecalis* bacteria

Fraction	Repetition of inhibition zone (mm)				Mean ±SD
	1	2	3	4	
2%	0	2.8	0	3.8	1.6
4%	0	2.7	2.8	2.75	2.0
6%	2.7	3.8	3.8	3.8	3.5
8%	2.8	3.9	3.8	3.9	3.6
CHX 0,2%	4.0	4.1	4.9	4.9	4.4
Methanol 96%	0	0	0	0	0

It can be seen that the 8% concentration has the largest inhibition zone diameter of 3.6



mm and the smallest at 2% concentration of 1.6 mm. The 4% and 6% concentrations produced inhibition zone diameters of 2.0 mm and 3.5 mm, respectively. The positive control of *chlorhexidine* obtained the diameter of the inhibition zone of *Enterococcus faecalis* bacterial growth of 4.4 mm, while the negative control of 96% methanol had no antibacterial activity.

Table 2. Result of normality test with *Shapiro-Wilk*

Group	Sig
2%	<0.05
4%	<0.05
6%	<0.05
8%	<0.05
K+	>0.05

Note: * (p>0.05)

In the normality test, a p-value of <0.05 was obtained, indicating that the data were not normally distributed.

Table 3. The result for the variance homogeneity with *Levene's test*

Levene's test	Sig
8,699	<0.05

Note: * (p>0.05)

The results of the variance homogeneity test with *Levene's test* obtained a p value <0.05, so it can be said that the data in this study are not homogeneous. Based on the normality and homogeneity tests, where the data is proven by the distribution of data is not normal and not homogeneous, then the *Kruskal-Wallis* nonparametric test is carried out with the provisions if the Sig value <0.05.

Table 4. Result of *Kruskal-Wallis* test

Variables	Sig
Zone of inhibition of <i>Enterococcus faecalis</i> bacteria	<0.05

Note: *Significant (p<0.05)

Based on the results of the *Kruskal-Wallis* non-parametric test, the sig value was 0.003<0.05, which means that the treatment tested had a significant effect on the inhibition zone of *Enterococcus faecalis* bacteria.

Table 5. Result of *Mann-Whitney* test

Group	Comparison concentration between group	Sig
K-	2%	>0.05
	4%	<0.05
	6%	<0.05
	8%	<0.05
	K+	<0.05
2%	4%	>0.05
	6%	>0.05
	8%	>0.05
	K+	<0.05
4%	6%	>0.05
	8%	<0.05
	K+	<0.05
6%	8%	>0.05
	K+	<0.05
8%	K+	<0.05

Notes: *Significant (p<0.05)

Based on the *Mann-Whitney* test results, there is a significant difference between K- and K+, 4% concentration with 8% concentration because the sig value is <0.05. Furthermore, at 2% concentration with 4%, 6%, and 8% concentrations, 4% concentration with 6% concentration, and 6% concentration with 8% concentration, there was no significant difference.

DISCUSSION

Based on the results of Table 1, the highest antibacterial activity was observed at a concentration of 8% (3.6 mm) to inhibit *Enterococcus faecalis* bacteria, and a small concentration that can inhibit *Enterococcus faecalis* bacteria is at a concentration of 2%.



According to Davis and Stout (1971), the strength of antibacterial power is divided into several criteria: weak (inhibition zone <5 mm), moderate (inhibition zone 5-10 mm), strong (inhibition zone 10-20 mm), and very strong (inhibition zone >20 mm). Based on these criteria, the antibacterial power of the ethyl acetate fraction of gletang plants (*Tridax procumbens* L.) against *Enterococcus faecalis* bacteria with concentrations of 2% (1.6 mm), 4% (2.0 mm), 6% (3.5 mm), and 8% (3.6 mm) was included in the weak category in inhibiting the growth of *Enterococcus faecalis* bacteria.²⁰ The size of the ability to inhibit the growth of the tested bacteria is influenced by the concentration of an ingredient that functions as an antibacterial. According to Imansyah *et al.* (2022), the difference in the diameter of the inhibition zone of each concentration is due to differences in the amount of active substances contained in that concentration; the greater the concentration, the greater the compound that acts as an antibacterial in the gletang plant fraction.

Ethyl acetate fractionation of gletang plants (*Tridax procumbens* L.) in inhibiting *Enterococcus faecalis* bacteria is higher at a concentration of 8%.^{19,20} This is due to the concentration of bioactive compounds that are sufficient to provide a synergistic effect on the antibacterial mechanism of action, this is in line with the research results of Sharma *et al.*, 2020 antibacterial activity of secondary metabolites such as flavonoids and terpenoids depends on concentration. At low concentrations, the amount of active compounds is not enough to effectively inhibit bacterial growth. Conversely, at high concentrations, these metabolites reach an effective threshold that allows significant inhibition of microorganisms.

Based on the results in Table 4, obtained a significant ($0.003 < 0.05$), meaning that the growth of *Enterococcus faecalis* bacteria is influenced by the ethyl acetate fraction of gletang plants (*Tridax procumbens* L.) with concentrations of 2%, 4%, 6% and

8%.^{19,20} This is due to the influence of several factors, including the results of the test of secondary metabolite compounds on the ethyl acetate fraction, which is known to contain flavonoids, terpenoids, saponins, and tannins with antibacterial properties.¹⁸

The results in Table 5. showed that at 2 %, 4 %, 6 %, and 8% concentrations, 4% and 6% concentrations, and 6% and 8% concentrations, there was no significant difference. This is due to the difference in active substances contained in the concentration of the fraction. The ethyl acetate fraction is a semi-polar solvent that can dissolve semi-polar compounds in cell walls. The ethyl acetate fraction is also known as a medium-polar solvent that is volatile, non-toxic, and non-hygroscopic. In line with research conducted by Kumar *et al.* (2020), the ethyl acetate fraction of *Tridax procumbens* L. contains active compounds such as flavonoids, tannins, saponins, alkaloids, steroids, terpenoids, and polyphenols that have potential as antibacterials. Tannins inhibit bacteria by denaturing bacterial cell proteins, inhibiting the function of cell membranes to interfere with the intercellular transport process, and disrupting bacterial growth by inhibiting nucleic acid synthesis.¹³ Saponins work as antibacterials by inhibiting the function of microbial cell membranes, forming complex compounds with cell membranes through hydrogen bonds, thus destroying the permeability properties of cell walls, causing cell detachment and cell death.¹⁴

CONCLUSION

The higher the concentration of the ethyl acetate fraction, the better the bacterial inhibition.

REFERENCES

1. Subrata A, Prahasti AE, Iskandar BO. Influence of two root canal obturation techniques with resin-based sealer to *Enterococcus faecalis*

- penetration. *J Indones Dent Assoc.* 2019;2(1):21. doi:10.32793/jida.v2i1.358.
2. Tamara R, Rochyani L, Budi Teguh P. Daya hambat ekstrak teripang emas (*Stichopus hermanii*) terhadap bakteri *Enterococcus faecalis*. 2015;9(1).
 3. Sofiani E, Mareta AD. Perbedaan daya antibakteri antara klorheksidin diglukonat 2% dan ekstrak daun jambu biji (*Psidium guajava* Linn) berbagai konsentrasi terhadap *Enterococcus faecalis*. *Insisiva Dent J.* 2014;3(1):30-41.
 4. Sayekti S, Subiwahyudi A, Prasetyo E. Perbedaan efektivitas daya antibakteri ekstrak daun mimba (*Azadirachta indica* A Juss) dibanding NaOCl 2,5% terhadap *Enterococcus faecalis*. *Conserv Dent J.* 2016;6:71-76. doi:10.20473/cdj.v6i2.2016.71-76.
 5. Tonini R, Salvadori M, Audino E, Sauro S, Garo ML, Salgarello S. Irrigating solutions and activation methods used in clinical endodontics: a systematic review. *Front Oral Health.* 2022;3:838043.
 6. Dea AHB, H EH, Susanto H. Uji efektivitas suspensi ekstrak daun songgolangit (*Tridax procumbens* L.) terhadap penurunan kadar asam urat pada tikus putih jantan. *J Farm Sains.* 2017;1(1):72-79.
 7. Ranjini SS, Abinash A, Sampath S, Yuvaneka S, Samiappan SC. *Tridax procumbens*-mediated silver nanoparticles synthesis against biofilm-forming pyogenic bacteria associated with wound. *3 Biotech.* 2026;16(2):66.
 8. Ingole VV, Mhaske PC, Katade SR. Phytochemistry and pharmacological aspects of *Tridax procumbens* (L.): a systematic and comprehensive review. *Phytomedicine Plus.* 2022;2(1):100199.
 9. Putri FE, Diharmi A, Karnila R. Identifikasi senyawa metabolit sekunder pada rumput laut coklat (*Sargassum plagyophyllum*) dengan metode fraksinasi. *J Teknol Ind Pertan Indones.* 2023;15(1):40-46. doi:10.17969/jtipi.v15i1.23318.
 10. Imansyah MZ. Uji daya hambat salep ekstrak etanol daun pandan wangi (*Pandanus amaryllifolius* Roxb.) terhadap bakteri *Staphylococcus aureus*. *J Kesehat Yamasi Makassar.* 2022;6(2):42-49.
 11. Sharma K, Kumar S, Pandey AK. Antibacterial activity of terpenoids in plant extracts: a review. *J Ethnopharmacol.* 2020;258:112874. doi:10.1016/j.jep.2020.112874.
 12. Kumar S, Prasad A, Iyer S, Vaidya S. Pharmacognostical, phytochemical, and pharmacological review on *Tridax procumbens* Linn.
 13. Salasa AM, Daswi DR, Arisanty A. Potensi antibakteri ekstrak etanol daun pegagan (*Centella asiatica* [L.] Urban) terhadap pertumbuhan *Enterococcus faecalis* dan *Citrobacter freundii*. *Media Farm.* 2022;18(1):67-73.
 14. Delina S, Arina Y. Uji aktivitas antibakteri dari fraksi daun kedondong bangkok (*Spondias dulcis* Forst.). *J Aisyiah Med.* 2022;7(2).
 15. Nurfatimah DA, Anwar R, Hardhani PR. Activity of water and ethanol *Syzygium myrtifolium* Walp. as antibacterial against *Aggregatibacter actinomycetemcomitans*. *DJKG.* 2025;19(1):1-7.
 16. Widyawati UA, Fadriyanti O, Situmeang B, Silaban S. Antibacterial activity test of different parts of gletang (*Tridax procumbens*) from West Sumatera, Indonesia. *Rasayan J Chem.* 2022;15(4):2382-2386.
 17. Wijayanti S, Pradana AF, Situmeang B, Prastiwi DA, Musa WJ. Microemulsion of methanol extract of *Tridax procumbens* flower and its antibacterial activity against *Streptococcus mutans* and *Enterococcus faecalis*. *J Beta Kim.* 2025;5(1):56-61.
 18. Situmeang B, Musa WJ, Bialangi N, Sriwijayanti S, Widiyanto H, Susvira D. Antioxidant and antibacterial properties of methanol extract of gletang flower (*Tridax procumbens*). *Biol Med Nat Prod Chem.* 2025;14(2):1085-1090.
 19. Ambulkar S, Ambulkar P, Deshmukh MP, Budhrani AB. Various dosage forms of *Tridax procumbens* and their antimicrobial activity against specific pathogens. *Indian J Forensic Med Toxicol.* 2020;14(4):6585-6588.
 20. Jangid T, Jain A, Bhardwaj GS, Jangir RN. A comprehensive review on traditional uses, phytochemical constituents, and pharmacological properties of *Tridax procumbens*. *J Phytopharmacol.* 2025;14(4):223-246.

