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The Differences of Antibacterial Activity of Gambier Leaf Extract (*Uncaria gambir Roxb.*) with Various Solvents Against *Enterococcus faecalis*

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Abstract

Introduction: Gambier leaves are herbal plants that have the potential to replace chlorhexidine to overcome root canal treatment failures where *Enterococcus faecalis* are commonly found. Various solvents such as n-hexane, ethyl acetate, ethanol, and distilled water extract from gambier leaves to increase antibacterial activity. **Purpose:** To determine the difference in antibacterial power of gambier leaf extract (*Uncaria gambir* Roxb.) with various solvents against *Enterococcus faecalis*. **Methods:** This study was an experimental in vitro laboratory investigation. The test group comprised extracts from gambier leaves using n-hexane, ethyl acetate, ethanol, and distilled water, all at a concentration of 10%, prepared through the graded maceration method. The antibacterial activity of these extracts was assessed using the disc diffusion method to determine the inhibition zone values. The results of the inhibition zones were analyzed statistically using one-way ANOVA and Post Hoc Tukey tests. **Results:** This study showed that a 10% ethyl acetate extract of gambier leaves had the highest average inhibition zone value of 16.10 mm, while a 10% distilled water extract of gambier leaves of 11.00 mm was the lowest. Statistically, there was a significant difference in the mean inhibition zone value between each group. **Conclusion:** The antibacterial power extract of gambier leaves (Uncaria gambir Roxb.) with a concentration of 10% with various solvents against Enterococcus faecalis showed a significant difference.

Keywords: Antibacterial; *Enterococcus faecalis*; gambier leaves; solvents; zone of inhibition

Introduction

Enterococcus faecalis is a facultative anaerobic Gram-positive cocci bacterium that often appears in cases of failed endodontic treatment.[1,2] The prevalence of *Enterococcus faecalis* involvement in cases post-endodontic treatment reaches 90%. Control of microorganisms in the root canal achieved by irrigation is critical to the success of root canal treatment.[3,4] Chlorhexidine (CHX) is the most commonly used irrigant. The weakness of chlorhexidine is that organic tissue cannot be dissolved and when used repeatedly over a long period it causes allergic reactions.[5,6] Currently, herbal ingredients are widely used because they produce fewer side effects from chemicals.

One of the herbal ingredients in Indonesia that has antibacterial activity is the gambier plant (*Uncaria gambir* Roxb.). Gambier has been used as a mouthwash, medicine for burns, diarrhea medicine, and as a mixture for betel nut. Alkaloids, flavonoids, steroids, terpenoids,

saponins, and phenolics are some of the secondary metabolites found in gambier leaves that have antibacterial properties.[7]

The solvents are used to extract polar and non-polar active compounds found in gambier leaves. Different solvents can dissolve active compounds with varying degrees of efficiency and selectivity. Water and ethanol can dissolve polar compounds. N-hexane can dissolve non-polar compounds. Polar and non-polar compounds can be extracted by ethyl acetate and other semipolar solvents.[8,9]

Novi et al. (2015) reported that 100% Cubadak gambir leaf aqueous extract could inhibit *Escherichia coli*, 90% Cubadak gambir leaf aqueous extract could inhibit *Salmonella typhimurium* and *Staphylococcus aureus*, and 80% Cubadak gambir leaf aqueous extract could inhibit *Bacillus cereus*.[10] Research by Putri et al. (2022) revealed that the growth of *Staphylococcus aureus* can be inhibited by ethanol extract of gambier leaves in concentrations of 10%, 20%, 30%, 40%, and 50%.[11] Based on research by Rini et al. (2019) gambier leaf extracted with various solvents can inhibit *Vibrio cholerae* bacteria. The results of this research show that 10% ethyl acetate, n-hexane and ethanol extracts of gambier leaves have produced inhibition zones of 18.7 mm, 14.3 mm, and 13.4 mm, respectively.[12]

Extracts of n-hexane, ethyl acetate, ethanol and aqueous from gambier leaves have antibacterial capabilities which have been reported in previous research. Therefore, the author wanted to examine the differences in the antibacterial power of gambier leaf extract (*Uncaria gambir* Roxb.) with various solvents on the growth of *Enterococcus faecalis*.

Methods

This in vitro laboratory experimental research was carried out from January to February 2024 in two places, the Chemical Engineering Laboratory of Sriwijaya State Polytechnic in Palembang, and the Microbiology Research Center Laboratory of the Faculty of Dentistry, Airlangga University. The subject used in this research was the bacterium *Enterococcus faecalis*. The sample size of research subjects was determined using the Federer formula. Based on sample calculations, the number of repetitions of each treatment was a minimum of 6 repetitions with the total sample in this study being 24. Glass equipment such as petri dishes, test tubes and other tools are sterilized in an oven at a temperature of 160-170°C for 1-2 hours.

Gambier leaf extract was obtained through a multilevel maceration process. First, the gambier leaves were thoroughly washed and then dried in direct sunlight for three days. After

drying, the leaves were ground using a blender and filtered to obtain a powder. Next, 200 grams of the gambier leaf powder were weighed and placed into a maceration bottle. Various solvents were added sequentially: n-hexane (non-polar), ethyl acetate (semi-polar), ethanol (polar), and finally distilled water (polar) at a 1:6 (w/v) ratio. The mixture was allowed to macerate for 72 hours, after which it was filtered.

The extraction process yielded both filtrate and residue. The filtrate was stored at room temperature, while the residue was re-extracted and filtered. Each filtrate obtained was then evaporated using a vacuum rotary evaporator until it formed a thick extract. This multilevel maceration process resulted in four 100% pure gambier leaf extracts that are thick and ready for use. Phytochemical tests on gambier leaf extract were carried out to ensure the presence of active compounds, namely alkaloids, flavonoids, saponins, phenolics, terpenoids, and steroids.

Test for alkaloids: Add 5 mL of chloroform and 5 mL of ammonia chloroform) to 0.2 grams of the sample, then shaken and filtered. Then 5 drops of 2 N H2SO4 were added then shaken and left until 2 layers were formed. The top layer was transferred into a test tube and then Mayer's reagent was added. The appearance of white precipitate indicates the presence of alkaloids. **Test for flavonoids**: Add a few drops of concentrated hydrochloric acid (HCl) to 0.2 grams of the sample, followed by 0.1 grams of magnesium powder. The appearance of a brick-red color indicates the presence of flavonoids. **Test for saponins**: Place 0.2 grams of the sample in a test tube, add 5 mL of distilled water, and shake vigorously. The formation of stable foam confirms the presence of saponins. **Test for phenolics**: Add 1-3 drops of 1% ferric chloride ($FeCl_3$) solution to 0.2 grams of the sample. A blackish-blue color indicates the presence of phenolics. **Test for terpenoids**: Dissolve 0.2 grams of the sample in 5 mL of chloroform, then add 5 mL of acetic anhydride and carefully add 2 mL of concentrated sulfuric acid (H_2SO_4) along the test tube wall. A red, orange, or purple color confirms the presence of terpenoids. **Test for steroids**: Place 0.2 grams of the sample in a test tube, add 2 drops of chloroform ($CHCl_3$) solution and 3 drops of Liebermann-Burchard reagent. A color change from red to blue and then green confirms the presence of steroids. Dilute gambier extracts from various solvents to a concentration of 10%.

Mueller Hinton Agar (MHA) media was made by adding 34 grams of MHA to 1 L of distilled water, then heating until homogeneous. The media was sterilized by autoclaving at 120oC for 15 minutes. After that, 20 ml of MHA medium was poured into a petri dish. *Enterococcus faecalis* bacteria were taken using a sterile tube needle and placed into a test tube

to be cultured in Brain Heart Infusion Broth (BHI-B) media. After that, the reaction tube was stored in an incubator for 24 hours at 37oC. Next, the bacteria grown on BHI-B media were suspended in a tube containing 2 ml of 0.9% NaCl solution until the turbidity was equivalent to the 0.5 McFarland standard (1.5 x 108 CFU/ml).

The antibacterial power test of gambier leaf extract was done using the Kirby Bauer method with paper discs. A sterile cotton swab was inserted into the bacterial suspension until it was wet, then squeezed by pressing it against the inner wall of the test tube, then smeared evenly on the surface of the MHA media. Paper discs were soaked in gambier leaf extract in n-hexane, ethyl acetate, ethanol, and distilled water, each concentration of 10%. Petri dishes were kept in the incubator for 24 hours at 37oC. Measurement of the inhibition zone formed around the paper disc was done by measuring the vertical diameter (DV) and horizontal diameter (DH) in millimeters (mm) using a caliper.

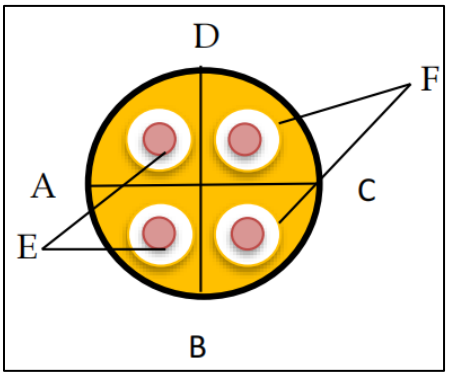

Figure 1. Inhibition zone calculation; A,C: Horizontal diameter; B, D : Vertical diameter, E: Extracted paper discs; F: Formed inhibition zone

The data obtained from this research was analyzed and processed using SPSS. Normality test was done using Shapiro-Wilk and the homogeneity test using Levene's test.

Results

Research has been conducted on the antibacterial properties of gambier leaf extract (Uncaria gambir Roxb.) using various solvents against Enterococcus faecalis. This study utilized qualitative phytochemical tests. The phytochemical results of the gambier leaf extract are presented in Table 1 below.

Table 1. Phytochemical Test Results of Gambir Leaves (*Uncaria gambir* Roxb.)

No	Phytochemical Test	N-hexane Extract	Etyl Acetate Extract	Ethanol Extract	Aquaeous Extract
1	Alkaloid	+	+	+	+
2	Flavonoid	-	+	+	+
3	Saponin	-	+	+	+
4	Fenolik	+	+	+	+
5	Terpenoid	-	-	+	+
6	Steroid	+	+	-	-

The results of the phytochemical test observations in Table 1 showed that all gambier leaf aqueous extracts with various solvents contain active compounds. Based on phytochemical tests, the extracts that contain the most active compounds are ethyl acetate, ethanol, and distilled water extract. The extract that contains the least types of active compounds is n-hexane extract. Gambir leaf extract contains the most active compounds, namely alkaloids, and phenolics, while the least active compounds are terpenoids and steroids.

The inhibitory zone test of gambier leaf extract (*Uncaria gambir* Roxb.) with various solvents with a concentration of 10% against *Enterococcus faecalis* was carried out using the disc diffusion method. The results of the inhibition test for gambier leaf extract were obtained by repeating the experiment six times (Figure 2), and then the diameter of the inhibition zone formed was measured using a caliper (Table 2).

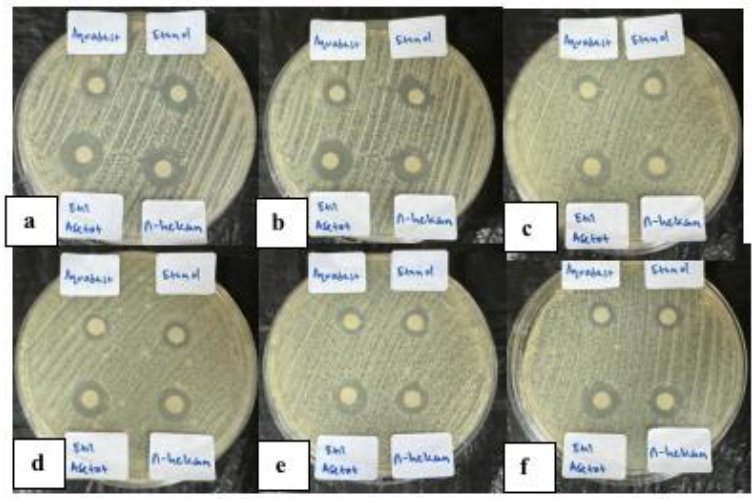

Figure2. Inhibition test results using the disc diffusion method using gambier leaf extract with n-hexane, ethyl acetate, ethanol and distilled water at a concentration of 10% after incubation for 24 hours (a) Repetition 1, (b) Repetition 2, (c) Repetition 3, (d) Repetition 4, (e) Repetition 5, (f) Repetition 6

Table 2. Results of measuring the diameter of the inhibitory zone of gambier leaf extract with various solvents against *Enterococcus faecalis*

No.	Group	Inhibition Zone Diameter (mm)						Mean (mm)
		Repetition						
		1	2	3	4	5	6	
1.	Gambir Leaf N-Hexane Extract 10%	15,80	14,60	15,40	14,80	14,80	15,20	15,10
2.	Gambir Leaf Ethyl Acetate Extract 10%	16,80	15,20	16,20	15,80	16,20	16,40	16,10
3.	Gambir Leaf Ethanol Extract 10%	13,20	13,05	13,15	12,80	13,05	13,15	13,06
4.	Gambir Leaf Aqueous Extract 10%	11,35	11,20	11,05	10,60	11,05	10,80	11,00

The data in Table 2 shows gambier leaf extract with various solvents, each in 10% concentration, is able to inhibit *Enterococcus faecalis*. The largest mean diameter of the inhibition zone was in 10% gambier leaf ethyl acetate extract, and the lowest was in 10% gambier leaf distilled water extract.

Inhibition zone diameter measurement data were statistically analyzed and processed using the SPSS program. Data for all treatment groups based on the results of the Shapiro-Wilk normality test were found to be normally distributed ($p>0.05$). Data for all treatment groups based on the results of the Levene's test of homogeneity were declared homogeneous ($p>0.05$).

The One-Way ANOVA test was conducted after confirming that the data was normally distributed and homogeneous. The results, with a significance value of $p<0.05$, indicated a significant difference in the inhibition of *Enterococcus faecalis* across all treatment groups (Table 3). Tukey's post-hoc test was then performed to confirm significant differences in inhibition zone values between the groups.

Table 3. Results of One-Way ANOVA test

	Gambir Leaf N-Hexane Extract 10%	Gambir Leaf Ethyl Acetate Extract 10%	Gambir Leaf Ethanol Extract 10%	Gambir Leaf Aqueous Extract 10%

Gambir Leaf N-Hexane Extract 10%		0,001	0,000	0,000
Gambir Leaf Ethyl Acetate Extract 10%	0,001		0,000	0,000
Gambir Leaf Ethanol Extract 10%	0,000	0,000		0,000
Gambir Leaf Aqueous Extract 10%	0,000	0,000	0,000	

Discussion

This research demonstrates that ethyl acetate, n-hexane, aqueous, and ethanol extracts from gambier leaves have strong antibacterial activity against *Enterococcus faecalis*, as evidenced by the formation of a strong inhibition zone. Different solvents can dissolve active compounds with varying degrees of efficiency and selectivity. Aqueous and ethanol can dissolve polar compounds. N-hexane can dissolve non-polar compounds. Polar and non-polar compounds can be extracted by ethyl acetate and other semipolar solvents.

The study also confirms that gambier leaf extracts from different solvents contain active compounds such as alkaloids, flavonoids, saponins, phenolics, steroids, and terpenoids. These findings align with research by Rini et al. (2019), which also identified these active compounds in gambier leaf extracts. [12] This supports the "like dissolves like" principle, which explains that solvents attract active compounds based on their polarity levels. [13]

The active compounds produced are responsible for antibacterial activity. Alkaloids inhibit the metabolism and synthesis of bacterial nucleic acids and damage cell membranes.[14] Flavonoids inhibit metabolism and nucleic acid synthesis and disrupt membrane function.[15] Saponins cause disruption of cytoplasmic membranes and membrane proteins and inhibit bacterial biofilms.[16] Phenolics can inhibit virulence factors such as enzymes and toxins, suppress biofilm formation, and interact with the cytoplasmic membrane.[17] Terpenoids inhibit oxygen absorption and oxidative phosphorylation causes limitation of respiration rates in bacteria.[18] Steroids involve the interaction of permeable cell phospholipid membranes with lipophilic compounds resulting in loss of membrane integrity and changes in membrane shape that can induce cell lysis.[19]

Aqueous and ethanol extracts, which are polar in nature, can effectively attract active substances such as alkaloids, flavonoids, saponins, phenolics, and terpenoids. Ethanol extracts demonstrate a better inhibition zone compared to distilled water extracts. This finding aligns

with the research by Salsabila et al., which indicates that ethanol extracts from papaya leaves yield a superior inhibition zone compared to distilled water extracts from the same leaves. The reason for this difference is that Ethanol is effective because its moderate polarity aligns well with the polarity of many active compounds, allowing it to extract these substances efficiently, whereas distilled water has a polarity level that is significantly different from these compounds. This variation in polarity can influence the concentration of active compounds obtained. [13]

Ethyl acetate and n-hexane extracts have better inhibition zones than ethanol and distilled water. Active compounds can influence antibacterial activity. Research by Yulianti et al. (2016) stated that the inhibitory power against bacteria depends on the type, amount or total level, and strength of the active compounds extracted because the active components produced are different from each solvent.[20] . Semipolar ethyl acetate can attract alkaloids, flavonoids, saponins, phenolics, and steroids. N-hexane, which is non-polar, can attract phenolic, steroid, and alkaloid active substances. Ethyl acetate extract of gambier leaves has a better inhibition zone value than n-hexane. Similar to research by Rini et al. which states that the ethyl acetate extract of gambier leaves has better inhibitory power than the n-hexane extract of gambier leaves.[12] This is due to the semipolar nature of ethyl acetate so that it can attract polar and non-polar compounds well. The ability to inhibit bacteria differs from each compound. The synergistic effects resulting from the active compounds differ depending on the morphology and type of bacteria.

Conclusion

There were differences in the antibacterial power of n-hexane extract, ethyl acetate, ethanol, and gambier leaf distilled water (*Uncaria gambir* Roxb.) at a concentration of 10% on the growth of *Enterococcus faecalis*. Ethyl acetate extract of gambier leaves has the highest inhibitory power compared to n-hexane, ethanol, and distilled water extracts of gambier leaves on the growth of *Enterococcus faecalis*.

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