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Research Report

The inhibitory activity of *Avicennia marina* leaf extract on *Enterococcus faecalis* as a root canal irrigation material

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ABSTRACT

Background: *Enterococcus faecalis is considered the most common cause of root canal failure and persistent infection. The standard irrigation solution is 5.25% NaOCl which has drawbacks, such as causing inflammation in the periapical area. New antibacterial agents from natural materials with similar antibacterial properties to chemicals have been developed. Avicennia marina contains flavonoids, saponins, tannins, and avicequinone, which can inhibit bacterial metabolism.* ***Purpose:*** *to determine the effect of Avicennia marina leaf extract with various concentrations on the antibacterial inhibitory power of Enterococcus faecalis as a root canal irrigation material.* ***Methods:*** *The samples were divided into six groups: K+ (5.25% NaOCl), K- (distilled water), P1 (7.5%), P2 (12.5%), P3 (17.5%), and P4 (22.5%). The leaves of Avicennia marina were extracted using ethanol maceration with a rotary evaporator to obtain a concentrated extract, which was then diluted with distilled water to the desired concentrations. The antibacterial effect was tested using the disk diffusion method by applying 0.01 ml of each test sample to sterile paper disks with a sterile micropipette.* ***Results:*** *Inhibition zones were observed as follows: K+ (21.67 mm), P1 (0 mm), P2 (10.50 mm), P3 (13.50 mm), and P4 (14.50 mm). Mann Whitney tests showed significant differences ($p < 0.05$) between K+ and P1, P2, P3 and P4, as well as between P3 and P4.* ***Conclusion:*** *Avicennia marina leaf extract had antibacterial effects against Enterococcus faecalis at concentrations of 12.5%, 17.5%, and 22.5%.*

Keywords: *Root canal irrigation, Sodium hypochlorite, Avicennia marina, Antibacterial, Enterococcus faecalis*

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INTRODUCTION

Endodontic treatment or root canal treatment is a treatment that is done by taking pulp tissue in the pulp chamber and root canal of teeth infected by bacteria. The primary purpose of endodontic therapy is to disinfect, clean, shape and obturate the root canal system and as well as eliminate reinfection.[1] Bacterial elimination is crucial for a successful endodontic therapy.[2] *Enterococcus faecalis* bacteria are found in the root canal isolated from endodontic infections in failed endodontic treatment.[3,4] *Enterococcus faecalis* is a bacterium that survives within dead organic tissues, and most commonly found in deep caries and root canal infections.[5,6] This bacteria can survive disinfection and responsible for periapical lesion formation.[2]

Root canal irrigation is used to clean the root canal from food residue in the oral cavity, organic tissue, microorganisms, pulp tissue residue, inorganic tissue, and non-pathogenic bacteria.[7] Many researches have studied antimicrobial activity of certain maerials.[8-10] Previous researches have studied natural materials for antibacterial activities for use in endodontics.[11-13] The most commonly used golden standard material as a root canal irrigation material is sodium hypochlorite (NaOCl). Sodium hypochlorite has a toxic effect when it comes into contact with the tissue at the tip of the tooth root, it can cause pain stimulation quickly in a matter of 2-6 minutes, soft tissue enlargement, enlargement spreading around the face area including the cheeks, the area around the eyes and lips and causing bruising on the skin surface due to direct trauma to the soft tissue.[14]

Avicennia marina is a species of mangrove plant that has a complex anatomical structure.[15] This plant is abundant in Indonesia with its vast waters and tropical climate, an ideal

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place for mangrove plants to grow. Many types of mangrove plants are traditionally used by coastal communities.[16] *Avicennia marina* leaves have many benefits and contain various bioactives, one of which is the active compounds contained in the *Avicennia marina* plant such as alkaloids, flavonoids, tannins and phenolic compounds.[17] In dentistry, the use of mangrove leaves and ascorbic acid combination gives good results in wound healing and can be developed as an alternative drug that is efficient, economical and safe. The content of active ingredients in the water extract of *Avicennia marina* mangrove leaves, ethanol extract of *Avicennia marina* mangrove leaves, and their combination with ascorbic acid were effective in increasing fibroblast cell proliferation.[18] Mangrove leaf extract (*Avicennia marina*) at a concentration of 7.5% can produce an optimal inhibitory effect on the growth of *Streptococcus mutans* bacteria with a diameter of 0.283 mm. Ethanol extract of *Avicennia marina* leaves with concentrations of 7.5%, 12.5%, 17.5%, 22.5% has been proven to inhibit *Streptococcus mutans* bacteria and *Candida albicans* adhesion.[19]

MATERIALS AND METHODS

This study is a laboratory experiment studying the inhibitory activity of *Avicennia marina* leaf extract against *Enterococcus faecalis* bacteria. With a research design of post-test only control group. The research objects were divided into 6 groups. The research design in group K- was the group that was not given treatment, K+ as a positive control using 5.25% NaOCl and groups 1, 2, 3, and 4 were treatment groups given *Avicennia marina* leaf extract with different concentrations of 7.5%, 12.5%, 17.5%, 22.5%.

The first procedure was sterilization of equipments and materials used in the study in an autoclave for 15 minutes, then preparation of test materials for *Avicennia marina* leaf extract was done using the maceration extract method, followed by the preparation of bacterial media, then the treatment of samples with different concentrations of 7.5%, 12.5%, 17.5%, 22.5%. *Avicennia marina* leaves were cut into small pieces, sun dried, and then extracted with ethanol 70% solvent, filtered and filtrate was evaporated with a rotary vacuum at a temperature of 40°C and 60 rpm for 6 hours, the extract was made into concentrations of 7.5% 12.5% 17.5%, 22.5% and K+ concentration of 5.25%, the filter paper was dipped for 10 seconds in MHA agar.

The *Enterococcus faecalis* bacterial media was processed by incubating the bacteria at 37°C for 24 hours and *Enterococcus faecalis* suspension was made in liquid BHI equalized with 0.5 Mc Farland solution. Inoculation of bacteria on MHA agar media, Incubation for 24 hours at 37°C, measurement of inhibition in millimeters and continued with data analysis. Furthermore, the antibacterial test method and the last measurement of the inhibition zone. The antibacterial test used in the antibacterial activity test is agar diffusion. The diffusion method determines the sensitivity of the test microbes to antimicrobial agents.[20]

The working principle of the diffusion method is by diffusing the compound that inhibits bacterial growth into a solid medium with the test microbe having been transferred directly from the microorganism culture to the inoculation needle.[21] Antibacterial activity testing of the agar diffusion method by measuring and observing the diameter of the clear zone formed around the disc containing antimicrobials that have been transferred from the culture for microbial growth. The advantages of the diffusion method can be done easily without using special tools and include greater maximum capabilities in selecting the drugs to be used.[20]

Statistical data analysis was conducted. Normality test was done using the Shapiro-Wilk test because the number of samples used was less than 50. The data obtained was not normal, because the data was not normally distributed ($p < 0.05$), so non-parametric testing was carried out and then testing was carried out using the Kruskal-Wallis hypothesis test and groups that had significant differences were then analyzed by the Post Hoc test with Mann-Whitney.

RESULTS

This study examines how effective *Avicennia marina* leaf extract in various concentrations is to inhibit the growth of *Enterococcus faecalis* bacteria. The results of the study were analyzed using a descriptive method, by looking at the average and standard deviation of each treatment group of *Avicennia marina* leaf extract concentration. This data can then be further tested using statistical tests with the SPSS 24 (IBM Corporation, New York, USA) program to prove the research hypothesis.

Table 1 shows that the average effect of *Avicennia marina* leaf extract with various concentrations on the antibacterial inhibitory activity of *Enterococcus faecalis* is shown in treatments P2, P3 and P4. Figure 1 shows the diameter of inhibition zone of *Avicennia marina* against *Enterococcus faecalis* on agar media. Based on table 2, it is known that the results of the normality test using the Shapiro-Wilk test show that the $p<0.05$, which indicates that the data is not normally distributed.

Table 1. The average inhibition zone of Avicennia marina leaf extract on Enterococcus faecalis in various concentrations

Groups	Replication (n)	Mean (mm)	Standard Deviation
K-	6	0	0
K+	6	21.3167	0.15706
P1	6	0	0
P2	6	10.2250	0.12942
P3	6	13.1750	0.12942
P4	6	14.7750	0.11292

The Kruskal Wallis test resulted p=0.001, which can be concluded that there is a significant difference between the control group and all treatment groups. Based on the results of the data, the statistical test can be continued with the Mann Whitney Test. Table 3 shows the results of comparison between groups. It can be said that there is a significant difference between the control group and the treatment group.

DISCUSSION

This in vitro true experimental laboratory study obtained the inhibition zone of *Avicennia marina* leaf extracts in millimeters (mm) compared to testing with 5.25% NaOCl concentration as positive control and found that there were inhibitory activities in certain concentrations against *Enterococcus faecalis* bacteria which showed antibacterial properties. Sodium hypochlorite (NaOCl) has antibacterial properties which interferes with oxidative phosphorylation and other membrane-related activities, and also works quickly with an inhibitory effect on mitochondrial function and bacterial DNA synthesis.[20] Sodium hypochlorite (NaOCl) can even kill bacterial biofilms. In the study, the effect of Sodium hypochlorite (NaOCl) with a concentration of 5.25% on *Enterococcus faecalis* bacteria was obtained by a clear zone measured with a caliper of 21.67 mm. This large inhibitory power can be due to Sodium hypochlorite (NaOCl) being able to oxidize peptide bonds in cell membranes which is high due to the formation of hypochlorous acid and the release of chlorine compounds which are very bactericidal.[22] This hypochlorous acid is very effective in inhibiting bacterial growth.[23]

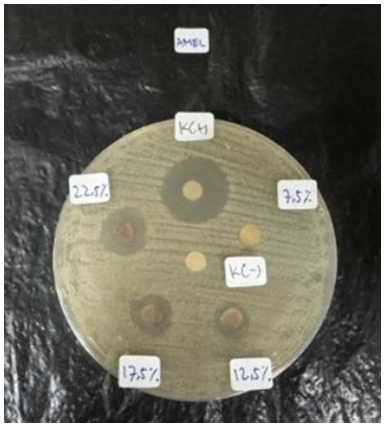

Figure 1. Diameter of inhibition zone of *Avicennia marina* against *Enterococcus faecalis* on agar media.

Table 2. Normality of data

Groups	Significance
K+	0.309
K-	0.000
P1	0.000
P2	0.692
P3	0.272
P4	0.580

In the treatment of 7.5% *Avicennia marina* leaf extract, no bacterial inhibition zone diameter was obtained, while 12.5% *Avicennia marina* leaf extract obtained a clear zone diameter with an average of 10.50 mm, the 17.5% *Avicennia marina* leaf extract obtained a clear zone diameter with an average of 13.50 mm, while the 22.5% *Avicennia marina* leaf extract obtained a clear zone diameter with an average of 16.50 mm. In comparison with negative control, the 7.5% *Avicennia marina* showed no inhibition while 12.5%, 17.5% and 22.5% concentrations of *Avicennia marina* showed inhibition against *Enterococcus faecalis*. Phytochemical studies on the roots and leaves from Jeddah, Saudi Arabia, showed higher compound content in the roots. The roots contain 19.7% total phenolics and 8.8% flavonoids, while the leaves contain 9.5% phenolics and 10.9% flavonoids. The root extract showed significantly higher levels of phenolics (394.8 mg/L) and flavonoids (175.9 mg/L) than the leaf extract (190.8 mg/L and 21.7 mg/L, respectively). Both roots and leaves showed significant antioxidant and antibacterial activities, with the leaf extract being more effective in inhibiting Enterococcus faecalis bacteria with an inhibition zone of 12 mm.[24] In the conducted study, there was inhibition at a concentration of 12.5%. The only part of the plant that is effective against VREF (Vancomycin-Resistant Enterococci) is the ethanol extraction of *Avicennia marina* from the leaves, and the highest inhibition zone is found in the 100% concentration leaf extract with an inhibition zone of 14.5 ± 0.7 mm, and the 50% concentration leaf extract with an inhibition zone of 10.5 ± 0.7 mm, increasing the concentration of *Avicennia marina* extract significantly increases the inhibition. The addition of mangrove extract showed that more mangrove extract significantly (p 0.05) increased the diameter of the inhibition zone against *Enterococcus faecalis*.[25]

Based on the results of the study, treatment with a concentration of 7.5% *Avicennia marina* leaf extract, no inhibition zone of *Enterococcus faecalis* bacteria was obtained. This is because the antibacterial compound in the

Table 3. Mann Whitney test result

Groups	K-	P1 (7.5%)	P2(12.5%)	P3(17.5%)	P4(22.5%)
K+	0.002*	0.002*	0.004*	0.004*	0.004*
K-	-	1.000	0.002*	0.002*	0.002*
P1	-	-	0.002*	0.002*	0.002*
P2	-	-	-	0.004*	0.004*
P3	-	-	-	-	0.004*

*Significant

Avicennia marina leaf extract is too small. Antibacterials generally work by damaging bacterial cell walls, inhibiting protein and nucleic acid synthesis. However, its effectiveness is influenced by various factors, including the type of bacterial species.[26] The pH, as important as temperature and humidity, has a significant effect on bacterial growth. Bacteria grow optimally in the pH range of 4-9, with an ideal pH of 6.5-7.5.[27] Concentrations of *Avicennia marina* at 12.5%, 17.5% and 22.5% in this experimental study obtained inhibition zone diameters of more than 10 mm, so it can be interpreted that the antibacterial inhibition activity of *Avicennia marina* leaves with concentrations of 12.5%, 17.5% and 22.5% is classified as strong. Inhibition zone diameters >10 mm are strong, 5-10 mm are in the medium and weak categories.[28] *Avicennia marina* leaf extract with concentrations of 12.5%, 17.5%, and 22.5% with a range of inhibition zone diameters of 10–20 mm is categorized as strong and does not cause harmful effects.[21] In the treatment of *Avicennia marina* leaf extract, the antibacterial inhibitory activity of *Enterococcus faecalis* was obtained due to the presence of flavonoids, saponins, trapenoids, and avicequinone. Phytochemical screening of bioactive compounds has been isolated and characterized from mangrove plants. These are included in the categories of glycosides, tannins, terpenes, steroids, naphthoquinones, alkaloids, and flavonoids. Due to the presence of various levels of bioactive compounds, research interest in mangrove plants for their therapeutic activities, including antimicrobial effects, continues to increase.[29]

The antibacterial activity of *Avicennia marina* leaves in this study could not exceed the antibacterial inhibitory activity of sodium hypochlorite (NaOCl). Research conducted by giving ethanol extract of *Avicennia marina* leaves at a concentration of 7.5% did not show any inhibitory power. This is in line with study conducted by Dinar *et al*. (2025) which study the effectiveness of *Avicennia marina* mangrove leaf extract on the growth of *Enterococcus faecalis*.[30] The test method used was a modification of Kirby-Bauer using wells. The samples were divided into four groups, each given a concentration of 60%, 70%, 80%, 90%, positive control (2.5% NaOCl), and negative control (aquadest). In that study, the average results of each treatment group were 60% concentration of inhibition zone 14 mm, 70% concentration of inhibition zone 15.9, 80% concentration of inhibition zone 16 mm, 90% concentration of inhibition zone 17.3 mm. The results of the normality test showed p> 0.05 which means the data was normally distributed. The one-way ANOVA test showed p <0.05 which means there was a difference in each treatment. The Tukey's HSD test showed a significant difference between treatments. The conclusion of that study was that *Avicennia marina* mangrove leaf extract inhibits *Enterococcus faecalis* bacteria at concentrations of 60%, 70%, 80%, and 90%, especially at a concentration of 90%.[31] The content of *Avicennia marina* leaf extract is known to have an antimicrobial effect, namely by forming a complex compound against extracellular proteins so that bacterial integration is disrupted.[32] Flavonoids have antibacterial effects with three mechanisms: inhibiting nucleic acid synthesis, damaging cell membranes through disruption of cell wall permeability and protein binding, and inhibiting the energy metabolism of oxygen use by bacteria and the ability of bacterial movement. Avicequinone also shows potential as an antibacterial with significant antiproliferative and cytotoxic activity.[33]

In conclusion, *Avicennia marina* extract with a concentration of 7.5% had no inhibitory effect on *Enterococcus faecalis* bacteria. There was antibacterial inhibition at concentrations of 12.5% 17.5% and 22.5%. The antibacterial inhibition of *Avicennia marina* leaf extract against *Enterococcus faecalis* bacteria was greatest at a concentration of 22.5%.

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