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THE ANTIBACTERIAL EFFECT OF ROSEMARY (ROSMARINUS OFFICINALIS L.) ON ENTEROCOCCUS FAECALIS BACTERIA A...

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THE ANTIBACTERIAL EFFECT OF ROSEMARY (ROSMARINUS OFFICINALIS L.) ON ENTEROCOCCUS FAECALIS BACTERIA AS AN ALTERNATIVE FOR ROOT CANAL IRRIGATION

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ABSTRACT

Background: Root canal treatment is one of the endodontic treatments to preserve teeth with bacterially infected pulp tissue. Root canal irrigation is one of the most important processes to eliminate bacteria in root canals. In the root canals of teeth with failed endodontic treatment, Enterococcus Faecalis bacteria were found, so that alternative irrigation materials were needed that were more effective.
Purpose: The aim of this study was to explain the effect of the antibacterial power of rosemary (Rosmarinus officinalis L.) on Enterococcus faecalis bacteria.
Methods: The samples tested were 24 in the form of Enterococcus faecalis bacteria culture in BHI agar media in petri dishes. This research uses the well method. Each 1 petri dish was divided into 4 wells and each was given rosemary extract (Rosmarinus officinalis L.) at 5% and 10% concentrations, as well as a positive control (NaOCl 2.5%). Bacteria were grown and incubated, then diluted with 0.5 McFarland standard. (1.5X108 bacteria permilliliter).
Results: The average inhibitory power of Enterococcus faecalis in rosemary (Rosmarinus officinalis L.) extract at 5% concentration was 16.7 mm and at 10% concentration was 18.8 mm, while in 2.5% NaOCl solution it was 8 mm. The hypothesis test has a value of $p = 0.000$ ($p < 0.05$).
Conclusion: Rosemary extract (Rosmarinus officinalis L.) has a more effective inhibition of Enterococcus faecalis bacteria.

Keywords: Rosmarinus Officinalis L., Enterococcus Faecalis, Root Canal Treatment, Antibacterial

1. INTRODUCTION

Root canal treatment is a treatment carried out by removing infected pulp tissue from the pulp chamber and root canal, then filling it with root canal filling material to prevent re- infection. Root canal treatment consists of three stages, namely biomechanical preparation of the root canal or cleaning and shaping, sterilization, and root canal filling. Pasril & Yuliasanti (2014) This can be achieved if the periodontal tissue is healthy or can be rehabilitated. The state of the crown of the tooth that allows for the creation of a good final restoration can also affect the success of root canal treatment. Expert knowledge and skills mastered as well as adequate tools and materials will also greatly determine the final outcome of root canal treatment. The outcome of root canal treatment should be predictable. Pulp and periapical disease are mostly caused by microorganism infection. Other factors that can exacerbate this situation include root perforation, broken instruments, excess root canal filling material and poor root canal filling technique. These things are not significant in causing root canal treatment failure when compared with microorganism infection. Removal of microorganisms from the root canal is a very important part of root canal treatment. Prasetia & Abidin (2016)

Enterococcus faecalis (E. faecalis) is a gram-positive facultative anaerobic bacteria species commonly found in cases of failure of endodontic treatment. Saatchi et al. (2014), Zancan et al. (2018) The presence of Enterococcus faecalis bacteria is able to colonize or adhere to protein surfaces and form biofilms on dentin walls. Enterococcus faecalis has a role of 80-90% against root canal infections. Gelatinase and hyaluronidase which are also enzymes in the bacterium Enterococcus faecalis cause tissue damage and are able to degrade the organic matrix of dentin.1 Resistant microorganisms such as E. faecalis are still a challenge to the success of root canal therapy, despite the application of various canal cleaning techniques and tools, as well as solutions. washers and antimicrobial agents. Akbari Aghdam et al. (2017) These bacteria have high resistance to high pH and saline concentrations and have a high capacity for biofilm formation, one of the most important virulence factors. Ghasemi et al. (2020)

Rosmarinus officinalis L. is one of the plants that has the potential to inhibit Enterococcus faecalis bacteria. Rosmarinus officinalis L. is a type of perennial woody plant originating from the Mediterranean region which can now be found and cultivated on all continents as an aromatic and ornamental plant. The leaves are usually used as a spice and medicinal purposes. The main constituents responsible for the pharmacological activity are 1,8-cineole (52.2%), camphor (15.2%), and α-pinene (12.4%). De Oliveira et al. (2017), Da Silva Bomfim et al. (2015) Natural extracts from the Lamiaceae family, such as rosemary, has been studied for its bioactive properties. Several studies have reported that rosemary extract exhibits bio-biological activities such as hepatoprotective, antifungal, insecticidal, antioxidant and antibacterial. The biological properties in rosemary are mainly due to the phenolic compounds, but it is important to consider that these biological properties depend on different aspects. Nieto et al. (2018), Laham & Fadel (2013)

Research conducted by Brito-Júnior et al. (2012), stated that the hydroalcoholic extract of the leaves of Rosmarinus officinalis can be used in endodontic practice because of its bactericidal effect that can inhibit the growth of Enterococcus faecalis. Brito-Júnior et al. (2012), Milyuhina et al. (2021) tested the Rosmarinus officinalis extract against Enterococcus faecalis, Staphylococcus aureus, and Eschericia colli bacteria, it was found that the Rosmarinus officinalis extract was effective against gram-positive and gram- negative bacteria. Milyuhina et al. (2021) In the scontrary, Sabzikar et al. (2020) revealed that rosemary ethanol extract at different concentrations did not show antifungal and antibacterial effects against C. albicans and S. aureus. Sabzikar et al. (2020) Research conducted by Vilela & De Oliveira Neves (2019) revealed that the hydroalcoholic extract of Rosmarinus officinalis in several different concentrations did not show any growth inhibition against the bacteria S. aureus, K. pneumoniaeand E. coli. L.) against Enterococcus faecalis bacteria. Vilela & De Oliveira Neves (2019)

2. MATERIALS AND METHODS

Rosemary (Rosmarinus officinalis L.) Extraction Methods

This was a post-test only design. This research begins with the selection of fresh and dried rosemary (Rosmarinus officinalis L.) and then pulverized into powder using a blender, Erlenmeyer flask containing alcohol and rosemary powder is placed into a shaker for 4-6 hours with 3 x 24 hours repetition while filtering and The filtrate is taken until the solvent gives effect. After getting the concentrated extract from rosemary, the next step is to make rosemary extract with a concentration of 5%, and 10% by diluting the concentrated rosemary extract using 5% DMSO as solvent.

Bacterial Culture

Enterococcus faecalis bacteria used in this study were bacterial culture stocks stored in eppendorf tubes obtained from the Indi Laboratory of Samarinda City. This bacterial culture stock was cultured on BHI media and incubated for 24 hours at 37ºC.

Antibacterial Activity

The antibacterial activity test method used to determine the inhibitory power of rosemary (Rosmarinus officinalis L.) extract to inhibit the growth of Enterococcus faecalis was the well method or Zone Will. This method is a method that is easy to do and relatively affordable in determining the sensitivity of bacteria to various contents. Suriawati et al. (2018)

Brain Heart Infusion Broth (BHIB) was used as a medium in this study because it is considered the best medium bacterial testing, especially for anaerobic bacteria such as Enterococcus faecalis bacteria used in this study. Tenda et al. (2017)

Mueller-Hinton agar media that had been prepared for each sample group were made 4 wells with a diameter of 6mm and added 100 microliters of rosemary extract (Rosmarinusofficinalis L.) with a concentration of 5%, rosemary (Rosmarinus officinalis L.) with a concentration of 10%, sodium hypochlorite (NaOCl) with a concentration of 2.5%as a positive control, and DMSO with a concentration of 5% as a negative control was poured into the well. Then the culture medium was heated in an incubator for 24 hours at 37ºC.

After the incubation time is complete, the inhibition zone or clear zone will be measured around the wells in each research sample group by using a large ratio of the outer diameter of the inhibition zone or clear zone using vertical and horizontal caliper. Take the average value of the diameter of the inhibition zone or clear zone to indicate the inhibition of the growth of Enterococcus faecalis bacteria.

3. RESULTS

The results of the study (Figure 1) showed that rosemary extract (Rosmarinus officinalis L.) with a concentration of 5% and rosemary extract (Rosmarinus officinalis L.) with a concentration of 10% had aninhibition zone around the well, indicating that both groups had inhibitory power against bacterial growth. Enterococcus faecalis. Based on the results of the Kruskal- Wallis non-parametric difference test that has been carried out, it is known that the difference in treatment has a significant effect on DDH Enterococcus faecalis, so to find out more precisely which group has the difference, it is necessary to proceed with a post-hoc test using the Mann- Whitney test. It is known that the treatments with significantly different

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results of DDH Enterococcus faecalis were rosemary extract 5% with K- and also with

K+, then rosemary extract 10% with K- and also with K+. Meanwhile, DDH Enterococcus faecalis rosemary extract 5% and DDH Enterococcus faecalis rosemary extract 10% did not differ significantly or could still be considered statistically the same. Likewise, DDH Enterococcus faecalis in the K- treatment and DDH Enterococcus faecalisin the K+ treatment were also not significantly different. Based on the results of the comparison test, because DDH Enterococcus faecalis in rosemary extract treatment gave a significant difference with the control treatment, it can be concluded that rosemary extract had a significant effect on DDH Enterococcus faecalis.

Figure 1

Figure 1 Research Results of Rosemary (Rosmarinusofficinalis L.) Extract at Concentrations of 5% and 10%, NaOCl 2.5%, and DMSO 5%.

Normality test was carried out using the saphiro-wilk normality test method. Based on the results of the normality test, it was known that the DDH data in the 5% and 10% rosemary extract groups produced p-value <0.05. This means that the assumption of normality of the data is not met. Whereas in the positive control group and the negative control group, the normality test could not be carried out, the statistic of the saphiro-wilk test could not be calculated because the data did not have variation (constant). So, the data is considered not normally distributed. Because the data does not meet the normal distribution, the different test using the parametric statistical method, namely the One-Way ANOVA method, cannot be carried out because the One-Way ANOVA requires normality in the data to be analyzed. So that the analysis of the difference test must be carried out using a non-parametric statistical method, namely the Kruskall-Wallis test because this test method does not require the assumption of data normality.

4. DISCUSSION

In this study sodium hypochlorite (Na OCl) concentration of 2.5% was used as a positive control group or comparison. has the advantages of having antimicrobial activity with a broad spectrum, the ability to fightanaerobic and facultative microorganisms

The antimicrobial effectiveness of sodium hypochlorite (NaOCl) is influenced by concentration, temperature, and time of application. Sodium hypochlorite (NaOCl) with a higher concentration has a greater ability to dissolve tissue. The duration of application of sodium hypochlorite (NaOCl) also affects the decrease in microhardness of root canal dentin, the ability to dissolve tissue, and the degradation of collagen in dentin in the root canal increases with the duration of application.19 The concentration of sodium hypochlorite (NaOCl) commonly used ranges from 0.5- 5.25%. Higher concentrations will have antimicrobial and tissue-destroying effects (toxic to tissues). Haapsalo & Wei Qian. (2019)

Sodium hypochlorite (NaOCl) concentration of 2.5% is the most suitable concentration for root canal treatment because of its low cytotoxicity and not irritating to periapical tissues but still exhibits bactericidal action and good dissolution time. Haapsalo & Wei Qian. (2019) Rosemary extract (Rosmarinus officinalis L.) contains bioactive compounds, such as phenolic diterpenes, triterpenes, phenolic acid, and flavonoids. These compounds have antibacterial properties to inhibit bacterial growth. Rosemary extract with concentrations of 5% and 10% had the inhibition of the growth of Enterococcus faecalis bacteria. This is due to the presence of bioactive compounds in rosemary extract which have antibacterial properties to inhibit bacterial growth with their respective inhibitory mechanisms. Other biological activities in rosemary include antioxidant, antimicrobial, anti- inflammatory, and anti-tumor. Sedighi et al. (2015)

Flavonoids work as antibacterial by inhibiting the synthesis of bacterial nucleic acids and inhibiting bacterial motility. Flavonoids work by interfering with the hydrogen binding of nucleic acids so that the DNA and RNA synthesis process is inhibited. Flavonoids can also prevent bacterial growth by disrupting cell membrane stability and bacterial energy metabolism. This instability occurs due to changes in the hydrophilic and hydrophobic properties of the cell membrane, so that the fluidity of the cell membrane decreases which results in disruption of fluid exchange in cells and causes bacterial cell death. Miftahhendarwati. (2014)

Figure 2

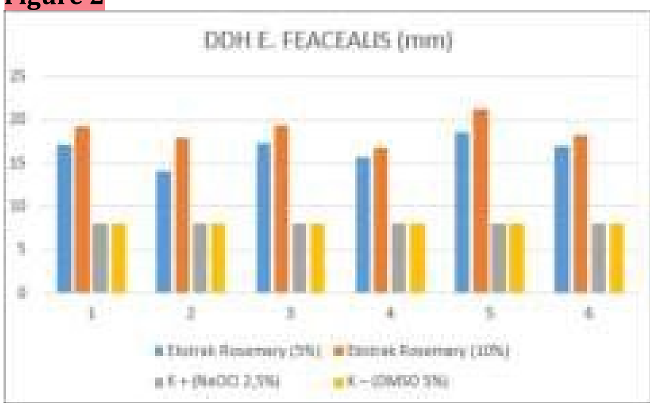

Figure 2 Enterococcus Faecalis DDH Data.

Table 1

Table 1 Mean, Standard Deviation and Median Data for Enterococcus faecalis DDH.

Rosemary	Enterococcus Faecalis (mm)						Mean	Median	Standard Deviation (SD)
	1	2	3	4	5	6			
Rosemary Extract (5%)	17.2	14.1	17.3	15.7	18.6	17	16.7	17.1	1.6
Rosemary Extract (10%)	19.2	18	19.4	16.8	21.3	18.3	18.8	18.8	1.5
K+ (NaOCl 2,5%)	8	8	8	8	8	8	8	8	0
K- (DMSO 5%)	8	8	8	8	8	8	8	8	0

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The first analysis is to do descriptive statistical analysis to determine the characteristics of the data from the results of the research that has been done. The descriptive statistics that were calculated were the mean, standard deviation and median of the data. The results of independent descriptive statistical analysis can be seen in Table 1 as follows. Based on the DDH test data, it can be seen that the highest DDH was in the 10% rosemary extract group. With a mean of 18.8 mm, a median of 18.8 mm and a standard deviation of 1.5 mm.

Table 2

Table 2 Normality Test Data

	P-Value
Rosemary Extract (5%)	0.010
Rosemary Extract (10%)	0.005
K+ (NaOCl 2,5%)	-
K- (DMSO 5%)	-

Based on Table 2, namely the results of the Kruskal-Wallis test, it is known that the resulting p-value is 0.000 which is smaller than the value of 0.05. It can be concluded that there is a significant difference in the growth inhibition of Enterococcus faecalis bacteria after being given rosemary (Rosmarinus officinalis L.) extract.

Based on the above discussion, the hypothesis in this study was accepted, namely that there was an inhibitory power of rosemary extract (Rosmarinus officinalis L.) at concentrations of 5% and 10% against the growth of Enterococcus faecalis bacteria. This research is still considered unsatisfactory because phytochemical tests were not carried out to find out more clearly the components of the bioactive compounds contained in rosemary (Rosmarinus officinalis L.) extract.

5. CONCLUSION

Rosemary extract (Rosmarinus officinalis L.) at concentrations of 5% and 10% had an inhibitory effect on the growth of Enterococcus faecalis bacteria. Rosemary extract (Rosmarinus officinalis L.) with a concentration of 10% had a higher but not significant inhibition compared to that of an extract of rosemary (Rosmarinus officinalis L.) with a concentration of 5% on the growth of Enterococcus faecalis bacteria. Rosemary extract (Rosmarinus officinalis L.) has a strong antibacterial inhibition as an alternative material for irrigation solutions in root canal treatment.

CONFLICT OF INTERESTS

None.

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