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Original Article

Potential of Hibiscus *(Hibiscus rosa sinensis L.)* Ethanol Extract as Root Canal Medicament Materials

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Abstract

Background : Enterococcus faecalis is the dominant bacteria found in root canals, especially in failure of root canal treatment. Therefore, antibacterial agents are required as a means to achieve complete disinfection of the root canal system. One of the natural ingredients that has been proven to have antibacterial properties is the ethanol extract of hibiscus *(H. rosa sinensis L.)*, so it has the potential to be used as a root canal medicament to inhibit growth and eliminate E. faecalis bacteria. This study was aimed to test the effectiveness of hibiscus *(H. rosa sinensis L.)* ethanol extract as a potential root canal medicament.
Methods : The study was conducted using a true experimental post-test only control group design in 6 treatment groups with various concentrations of hibiscus extract (100%, 50%, 25%, 12.5%, 6.25, and 3.125%) and a positive control group. (Brain Heart Infusion added *E. faecalis*) and negative (Brain Heart Infusion). The extract was then diluted to the concentration used and added with 1.5x108CFU/ml *E. faecalis* and then cultured using the spread method on NA media, incubated anaerobically at 37oC for 24 hours, then the colonies that grew were counted using a colony counter.
Results : The results showed that 100% concentration had the highest ability to inhibit the growth of E. faecalis bacteria (92.7%) with an average number of colonies growing of 4.4 x 102 CFU/ml. The minimum inhibitory concentration (MIC) was shown at a concentration of 50%, with an inhibiting ability of 90.17% and the average number of colonies that grew was 5.9 x103 CFU/ml. Kruskal Wallis statistical test showed a significant difference in the number of colonies ($p<0.05$).
Conclusion : Hibiscus extract with a concentration of 100% has the highest ability to inhibit the growth of E. faecalis bacteria and MIC is present at a concentration of 50%.

Keywords : *H. rosa sinensis L., E. faecalis*, root canal medicaments, MIC, inhibition ability

INTRODUCTION

People are starting to understand that treating aching teeth is a good idea to prevent tooth decay and even tooth loss.[1] The endodontic triad consists of preparation, shaping and cleaning of the root canals, as well as obturation or filling of the root canals which are the keys to successful endodontic treatment.[2] Endodontic treatment aims to maintain the tooth in the oral cavity as long as possible by filling the root canal and forming a good closure at the apical foramen of the tooth so that it cannot be penetrated by infectious fluids secondary to leakage of the periradicular tissue.[3] Endodontically treated teeth should be evaluated clinically and radiographically to ensure that the root canal treatment was considered successful and that the tooth is functional.[4]

Failure of endodontic treatment can be determined based on clinical and radiographic signs and symptoms found in teeth that have been treated with root canals.[5] Common factors leading to failure of endodontic treatment are inadequate root canal filling, filling leaks, untreated root canals, iatrogenic procedure errors, instrumentation complications such as perforation and persistence of bacteria in the root canal area such as isthmus, dentinal tubules, and bifurcation.[4] *E.faecalis* is a gram-positive bacterium which results in 70% of cases of canal treatment failure because it is able to invade, settle in the dentinal tubules and is resistant to antibacterials so that it can survive in the root canals in an alkaline pH environment.[6–9]

Calcium hydroxide is a root canal drug that is commonly used because it has antibacterial properties that work by diffusion from OH^- ions so that the pH is alkaline and does not support the environment for anaerobic bacteria in the root canals.[9–11] Calcium hydroxide has low solubility and diffusibility, so it cannot kill bacteria located in the isthmus, dentinal tubules, and bifurcations especially in *E. faecalis* and has a negative impact on periodontal tissue and is difficult to clean from the root canal walls.[12,13]

Alternative root canal drugs are needed to inhibit the growth of *E. faecalis*, so that the bacteria does not develop and cause secondary infections in the root canals. Root canal medications that are commonly used contain many synthetic chemicals that will have a negative impact, while herbal products are increasingly popular. Research in this regard continues to be carried out to find basic ingredients from traditional plants and materials that are non-toxic, biocompatible, and easily available in Indonesia's natural environment, which are expected to be used as substitutes for synthetic chemicals.[14]

Natural ingredients that are believed to have antibacterial properties are hibiscus *(Hibiscus rosa sinensis L.)*.[15] The results of phytochemical screening in previous studies stated that hibiscus leaf and flower extracts contain chemical compounds that play a role in inhibiting the growth of *E. faecalis* bacteria because they have active ingredients such as flavonoids and phenols which have significant inhibitory power.[16] Flavonoids can lyse bacteria because they have the ability to disrupt the integrity of cell membranes so that they can reduce the physiological activity of bacteria, while phenols work by damaging cell membranes and enzymes in bacteria. Another study found that high concentrations of the methanol extract of the hibiscus plant *(H. rosa sinensis L.)* showed strong activity against *Streptococcus mutans*.[17,18]

Based on the description above, it is known that hibiscus *(H. rosa sinensis L.)* has potential as a root canal medicament. In addition, hibiscus is also biocompatible with low toxicity, but no studies have tested the effectiveness of this plant against *E. faecalis* in root canals.[19] Therefore, researchers feel the need to conduct research on the antibacterial power of hibiscus *(H. rosa sinensis L.)* ethanol extract on the growth of *E. faecalis*, considering that this plant has the potential as a root canal medicament.

METHODS

The research was carried out at the Microbiology Laboratory of the Faculty of Medicine and the Diponegoro University Semarang Integrated Laboratory from October to December 2020. A true experimental study with a post-test only control group design was used as the type of study. The materials used in this study included ethanol extract of hibiscus *(H. rosa sinensis L.)* with the specified concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125% and the control group was a positive control containing BHIB *(Brain Heart Infusion Broth)* media with the addition of *E. faecalis* bacteria which had been adjusted to the Mc Farland 0.5 standard and the negative control only containing BHIB media. The *E. faecalis* bacteria is the result of culture from the Diponegoro University Microbiology Laboratory.

Hibiscus *(H. rosa sinensis L.)* obtained from the yard of the Faculty of Public Health UNDIP was then aerated until it reached a moisture content of <20% and extracted by maceration method using 70% ethanol solvent. The ethanol extract of hibiscus *(H. rosa sinensis L.)* with honey-like consistency was diluted (dilution) and various concentrations were obtained as the treatment group. *E. faecalis* seeding as much as 0.05 ml which had been standardized with Mc Farland 0.5 (1.5 × 108CFU/ml) was then added to the tube of the treatment and positive control groups, then labeled according to the concentration and incubated anaerobically at 37°C for 24 hours. Changes in turbidity in the treatment group were observed by comparison with the control group. Each tube in the treatment and control groups was taken 0.1 ml with a micropipette and planted in Nutrient Agar (NA) media using the spreading technique, then incubated

anaerobically at 37°C for 24 hours. The counting of growing colonies was then carried out using a colony counter and expressed as colony forming units (CFU/ml). Calculation of the number of sample replications refers to the Federer formula, so that it is obtained that each treatment group was replicated 4 times.

RESULTS

The data obtained in this study were primary data, namely the number of *E. faecalis* colonies that grew on NA media. Colonial growth of *E. faecalis* after incubation occurred in all treatment groups and positive control. The results of counting the number of colonies can be seen in Table 1.

Table 1 shows that the concentration of 100% has the smallest average number of colonies, namely 4.4 x102 CFU/ml, whereas at concentrations of 25% to 3.125% the average number of colonies Too Numerous To Count (TNTC) because it has > 300 colonies.

Calculation of MIC and MBC determination:
Concentration 100% =

$$\frac{44}{600} \times 100 = 7{,}3\% \longrightarrow 100\% - 7{,}3\% = 92{,}7\%$$

Concentration 50% =

$$\frac{59}{600} \times 100 = 9{,}83\% \longrightarrow 100\% - 9{,}83\% = 90{,}17\%$$

The Minimum Inhibitory Concentration (MIC) in this study was conducted to identify the antibacterial activity of the ethanol extract of hibiscus *(H. rosa sinensis L.)*. MIC is the smallest concentration of the experimental substance that can kill 90% of the bacteria from the average number of growing bacterial colonies. This study resulted in the average number of *E. faecalis* colonies growing at a concentration of 100% which was 4.4 x102 CFU/ml, while at a concentration of 50% it was 5.9 x103 CFU/ml. Based on these results, it is known that a concentration of 100% has the ability to inhibit the growth of *E. faecalis* by 92.7%, and a concentration of 50% has the

TABLE 1
The results of the descriptive analysis of counting the number of E. faecalis colonies

	Number of Bacterial Colonies (CFU/ml)							
Replication	**100%**	**50%**	**25%**	**12.5%**	**6.25%**	**3.125%**	**K+**	**K-**
1	$1x10^2$	$1.4x10^3$	TNTC	TNTC	TNTC	TNTC	TNTC	0
2	$3.6\ x10^2$	$5.1x10^3$	TNTC	TNTC	TNTC	TNTC	TNTC	0
3	$4.2\ x10^2$	$6.4\ x10^3$	TNTC	TNTC	TNTC	TNTC	TNTC	0
4	$5.4\ x10^2$	$6.2\ x10^3$	TNTC	TNTC	TNTC	TNTC	TNTC	0
Mean	$4.4\ x10^2$	$5.9\ x10^3$	TNTC	TNTC	TNTC	TNTC	TNTC	0

K+ = Positive control
K- = Negative control
TNTC = Too Numerous To Count.[20]

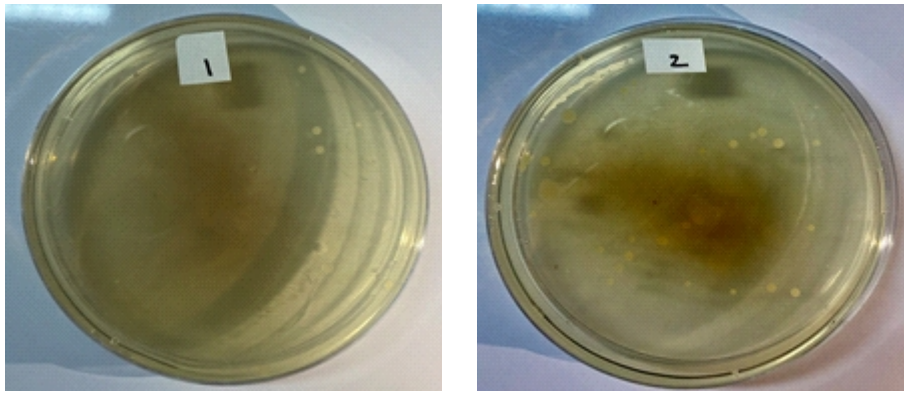

Figure 1. Colony growth of *E. faecalis* on NA media after 24 hours of incubation. (1) *E. faecalis* colony growth at 100% concentration; (2) Colony growth of *E. faecalis* at 50% concentration.

TABLE 2
The results of the Kruskall Wallis test for antibacterial activity of the ethanol extract of hibiscus *(H. rosa sinensis L.)*

Replication	Group	Mean Rank	p Value
Antibacterial power of ethanol extract of hibiscus *(H. rosa sinensis L.)*	100%	7.50	0.000*
	50%	9.50	
	25%	14.50	
	12.5%	18.50	
	6.25%	22.75	
	3.125%	26.25	
	K+	30.50	
	K-	2.50	

*Significant p<0.05

TABLE 3
Mann Whitney post hoc test results

	Group							
Group	**100%**	**50%**	**25%**	**12.5%**	**6.25%**	**3.125%**	**K+**	**K-**
100%	–	0.248	0.021*	0.021*	0.021*	0.021*	0.014*	0.014*
50%	0.248	–	0.021*	0.021*	0.021*	0.021*	0.014*	0.014*
25%	0.021*	0.021*	–	0.021*	0.021*	0.021*	0.014*	0.014*
12.5%	0.021*	0.021*	0.021*	–	0.021*	0.021*	0.014*	0.014*
6.25%	0.021*	0.021*	0.021*	0.021*	–	0.043*	0.014*	0.014*
3.125%	0.021*	0.021*	0.021*	0.021*	0.043*	–	0.014*	0.014*
K+	0.014*	0.014*	0.014*	0.014*	0.014*	0.014*	–	0.008*
K-	0.014*	0.014*	0.014*	0.014*	0.014*	0.014*	0.008*	–

*Significant p<0.05

ability to inhibit the growth of *E. faecalis* by 90.17%. Therefore, the concentration of 50% is determined as the MIC.

In addition to MIC, in order to identify the antibacterial power of the ethanol extract of hibiscus *(H. rosa sinensis L.)* can also be done by determining the Minimum Bactericidal Concentration (MBC). MBC is the smallest concentration of experimental material that can kill 99.9% of bacteria from the average number of bacterial colonies that have successfully grown. In this study, all test concentrations (100%, 50%, 25%, 12.5%, 6.25%, and 3.125%) still contained *E. faecalis* colony growth, so it can be said that the MBC could not be determined.

The results of the normality test carried out using Shapiro Wilk are intended to see the distribution of the data, and the data is categorized as normal if the p value is >0.05. In this study the significance values for concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125% respectively were 0.671; 0.122; 0.415; 0.360; 0.662; and 0.830. These results indicated that overall the data on the growth of the number of colonies of *E. faecalis* at all concentrations of the treatment groups had normal distribution data. Homogeneity test with Levenne's test obtains a significance value of 0.004 ($p > 0.05$), so it can be said that the data is not homogeneous. Therefore, in order to determine the antibacterial power of the ethanol extract of hibiscus *(H. rosa sinensis L.)* against *E. faecalis*, a

non-parametric Kruskall Wallis test was carried out and followed by the Mann Whitney post hoc test with a significant value of p<0.05.

The results of the Kruskal Wallis test in Table 2 showed that there was a significant difference in the number of *E. faecalis* colonies in the eight treatment groups (p <0.05), so that it was continued with the Mann Whitnney Post Hoc test to find out the differences between groups.

Table 3 shows that the concentrations of 100% and 50% have a p>0.05, which means that there is no significant difference in the number of *E. faecalis* colonies in the two groups. However, at concentrations of 25%, 12.5%, 6.25%, 3.125%, the positive control and negative control had p <0.05 which means there was a significant difference in the number of *E. faecalis* colonies.

DISCUSSION

The aim of this study was to determine the potency of hibiscus *(H. rosa sinensis L.)* ethanol extract as a root canal medicament, which showed a significant difference in the number of *E. faecalis* colonies (p<0.05). The results showed that the greater the concentration of the ethanol extract of hibiscus *(H. rosa sinensis L.)* the lower the average growth of *E. faecalis*. This happened because the greater the concentration of the ethanol extract of hibiscus *(H. rosa sinensis L.)* used, the greater the chemical compounds contained in the plant extract. The chemical compounds contained in the ethanol extract of hibiscus *(H. rosa sinensis L.)* are flavonoids, tannins, saponins, and terpenoids which are polar. These chemical compounds are secondary metabolites which are proven to have antibacterial properties and can be used to inhibit the growth and kill bacteria.[22]

The polar chemical compounds were extracted by maceration using 70% ethanol which was also polar. The use of this solvent is because polar solvents have the ability to bind and attract/dissolve polar and nonpolar chemical compounds contained in an extracted material better than nonpolar solvents which will only bind and attract/dissolve nonpolar compounds only.[23] Therefore, its use as a solvent is considered the best to be able to attract compounds in hibiscus *(H. rosa sinensis L.)* which have the potential as an antibacterial to inhibit the growth or kill *E. faecalis* bacteria.

E. faecalis has virulence factors that cause this bacterium to survive and have the ability to form colonization.[24] Extracellular surface protein (ESP) is a virulence factor that protects these bacteria from environmental stresses such as changes in pH, osmotic changes, and temperature. This ESP also plays a role in the exchange of ions such as cations, metals, and toxins. Chemical compounds that are positively charged will interact with the negatively charged *E. faecalis* so that the outer cell membrane is damaged and causes bacterial constituents to come out. However, the positive charge of these chemical compounds can be inhibited by the negatively charged ESP so that the bacteria can inactivate the antibacterial.[25,26]

Antibacterials are chemical compounds that have the property of lysing or inhibiting the growth of pathogenic bacteria with low toxicity to humans.[27] Pelczar and Chan (2008) suggested that the mechanism of action of antibacterials can be divided into bacteriostatic and bactericidal. Bacteriostatic is a chemical compound that can inhibit or suppress the growth of bacteria, while bactericidal is a chemical compound that can kill bacteria.[28] The results of the MIC and MBC tests using the dilution method showed that the ethanol extract of hibiscus *(H. rosa sinensis L.)* could only inhibit the growth of *E. faecalis*. This means that in this study, the extract was bacteriostatic, with an inhibitory power on the growth of *E. faecalis* bacterial colonies of 92.7% at an extract concentration of 100%, while a concentration of 50% had the smallest inhibition, namely 90.17%, so that this concentration can be said to be the MIC.

The results of the research that has been carried out are in line with research by Pangkuan *et al*., (2020) which revealed that hibiscus flower extract contains a relatively weak inhibitory effect on the growth of *Streptococcus mutans* bacteria.[17] Goering *et al*., (2013) suggested that bacteriostatic is an antibacterial property that can inhibit bacterial growth and is temporary (reversible). The bacteriostatic nature has a mechanism of action by inhibiting protein synthesis which temporarily binds to the ribosome of an organism. The bond of the organism's ribosomes can be released when its concentration and stability decrease, so that the antibacterial agent will release the ribosome again and cause the bacteria to grow again.[22] Chemical compounds lyse bacterial cells because they can damage the integrity of the bacterial cell membrane, causing bacterial cell lysis. Tannin compounds are able to inhibit bacterial growth by inhibiting enzyme production, interfering with enzymatic reactions and reducing calcium ions which have a role in the plasma coagulation process. Flavonoid compounds found in hibiscus flowers are antibacterial because they contain phenol groups which break down proteins and damage cell membranes thereby inhibiting bacterial growth. That is why the chemical compounds from the ethanol extract of hibiscus *(H. rosa sinensis L.)* can inhibit and suppress the growth of *E. faecalis* bacteria. However, it is still necessary to carry out toxicity tests and stability tests of the ethanol extract of hibiscus *(H. rosa sinensis L.)* in relation to its use as a root canal medicament.

CONCLUSION

The inhibition of the growth of *E. faecalis* bacterial colonies at a concentration of 100% ethanol extract of hibiscus *(H.*

rosa sinensis L.) was 92.7%, while a concentration of 50% had the smallest inhibition, namely 90.17%, so this concentration could be said to be the MIC. Therefore, this hibiscus ethanol extract has the potential to be used as a root canal medicament.

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