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ANTIBACTERIAL EFFECTIVENESS OF BEETROOT EXTRACT (Beta vulgaris L.) AGAINST Enterococcus Faecalis

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ABSTRACT

Endodontic treatment failure can be caused by Gram-positive bacteria, Enterococcus faecalis. Natural ingredients such as beetroot extract (Beta vulgaris L.) can be used to inhibit the growth of Gram positive bacteria. Beetroot extract (Beta vulgaris L.) has antibacterial properties because it contains active substances of flavonoids, phenols, tannins, saponins and alkaloids.To determine the antibacterial effect of beetroot extract (Beta vulgaris L.) against Enterococcus faecalis bacteria.This study was a quasi-experimental study consisting of five treatment groups, namely the beetroot extract group with concentrations of 10,000 µg/ml, 12,500 µg/ml, and 15,000 µg/ml, and 2% Chlorhexidine Gluconate as positive control, and Aquadest as negative control. Test the antibacterial effect using the well-diffusion method with Mueller Hinton Agar media. The repetition of the test for each group was 3 times. The media was incubated for 24 hours at 37ºC. After 24 hours, the diameter of the inhibition zone was calculated using a caliper in millimeters. Data were analyzed with the Kruskal-Wallis test and the Mann-Whitney test.There was a significant difference between all treatment groups ($p<0.05$). Only beetroot extract concentration of 10,000 µg/ml did not show an inhibition zone, while concentrations of 12,500 µg/ml and 15,000 µg/ml showed an inhibition zone. The diameter of the largest inhibition zone was at a concentration of 12,500 µg/ml, which was 2.33 ± 0.06 mm, but still smaller than the positive control, namely Chlorhexidine Gluconate 2%, which was 2.33 ± 0.06 mm.Beetroot extract (Beta vulgaris L.) can inhibit the growth of Enterococcus faecalis bacteria with the largest inhibition zone diameter achieved by a concentration of 15,000 µg/ml.

Keywords: Antibacterial effectiveness, Beta vulgaris L, Enterococcus faecalis, Zone of inhibition

1. INTRODUCTION

The majority of Indonesian citizens are still not aware of the importance of general body health, as well as concern for dental and oral health which will also have an impact on body health, the condition of healthy teeth and mouth is closely related to the overall level of body immunity.[1] A common occurrence in Indonesia is caries or cavities, according to Basic Health Research (Riskesdas) in 2018, the national prevalence was recorded at 88.8% of people experiencing caries.[2] Untreated caries can cause carious lesions to develop into pulp disease.[3]The most common pulp disease is caused by bacteria, which if left untreated can cause inflammation of the vital pulp resulting in pulp necrosis.[3] Pulp necrosis is a condition where the pulp is non-vital or dead, which if left unchecked will develop into periapical disease. One

of the treatments for pulp disease is endodontic treatment.[3]

Endodontic treatment is the removal of vital or non-vital pulp from the root canal and replacing it with filling material which aims to maintain the health of the tooth and the health of the periapical tissue so that it can continue to function in the dental arch.[3,4] In the endodontic treatment process there are three stages (Endodontic Triad) which include biomechanical preparation, irrigation, and hermetic root canal filling.[3] Each stage is a very important factor for the success of endodontic treatment, especially in the irrigation process which aims to eliminate microorganisms in infected root canals, the ideal irrigation must be antimicrobial and does not irritate host tissue.[3]

Based on research, it has been found that 90% of cases of endodontic treatment failure are caused by bacteria.[5] One of them is the Enterococcus faecalis bacteria with a prevalence that can reach 70%.[6.7] Enterococcus faecalis is a gram-positive, facultative anaerobic, cocci-shaped bacterium. Some irrigation solutions that are usually used are Sodium Hypochlorite (NaOCl), Chlorhexidine Gluconate 2%, and Ethylene Diamine Tetraacetic Acid (EDTA).8 Chlorhexidine Gluconate 2% and Sodium hypochlorite (NaOCl) are the gold standard for irrigation solutions because they have high antimicrobial capabilities, but has toxicity.[6]

Antibacterials are substances that can kill bacteria or prevent their growth.[9] Based on their source, antibacterials are classified into natural and synthetic substances. The use of natural ingredients can reduce the use of synthetic substances in medicine. Many plants are used to treat various diseases because they have antibacterial activity. One of the herbal plants that can be used as an antibacterial in the oral cavity is beetroot (Beta vulgaris L.).[10,11,12]

Beetroot contains bioactive pigments which are commonly used as natural food colorings, traditional medicines and cosmetics. Beetroot can inhibit the growth of gram-positive bacteria such as Streptococcus mutans, Staphylococcus aureus, and Escherichia coli, Salmonella typhimureum for gram-negative bacteria. 25,000 µg/ml, 50,000 µg/ml, 100,000 µg/ml have antibacterial activity against gram-positive Streptococcus mutans.[11] Research conducted by Hossam et al concluded that beetroot extract has strong antibacterial action and effectively stops the growth of gram-positive bacteria. compared to gram negative bacteria.[13]

2. METHODS

This research is an in vitro laboratory experimental study with a post-test only

with control group design, to determine the antibacterial properties of beetroot extract (Beta vulgaris L.) against Enterococcus faecalis bacteria. This research was carried out in two places, namely the Biochemistry Laboratory of the Faculty of Medicine, Sriwijaya University Palembang for making beetroot extract (Beta vulgaris L.) and the Inter-University Research Laboratory (PAU) of Gadjah Mada University for antibacterial testing against Enterococcus faecalis.

The sampling technique in this study used the consecutive sampling method, which means samples that met the research criteria and the number of samples was determined in the research, fresh beets, red to dark purple beets and beets in highland areas.5 kg of beets are washed with running water until clean. After that, cut the beets into small pieces and dry them in the oven at a temperature of 40°C for 24 hours and get 202g of dried beets. Once dry, the beets are ground into powder using a Philips blender until they become powder. Beetroot powder is made using the maceration method, which is soaked using 95% ethanol solvent in a ratio of 1:3. Then shake for 1 hour until homogeneous. Macerate the solution for 3x24 hours at room temperature and cover the extract tightly. Then filtrate the extract with 90mm filter paper to increase the effectiveness of the extract. The resulting extract phytrate was then concentrated in a rotary vacuum evaporator at a temperature of 50°C until a beetroot extract concentration of 100% was obtained.

The disc diffusion method was used to determine the inhibition zone of beetroot against Enterococcus faecalis bacteria. Mueller Hinton Agar (MHA) media was inoculated with Enterococcus faecalis ATCC 29212 using a sterile cotton bud. Each variation of beetroot extract concentration, namely 10,000µg/ml, 12,500µg/ml, and 15,000µg/ml, was saturated onto the disc. Then place the disc paper on the surface of the media. Then the press was incubated at 37°C in an incubator for 2 x 24 hours. The zone of inhibition is marked with a clear area as an indication of the sensitivity of the bacteria to the antibacterial agent used. The inhibition zone formed was measured in vertical and horizontal diameters in units (mm) using a caliper.

Gambar 4. Cara pengukuran diameter zona hambat.[54]

Keterangan :

Pengukuran 1 (mm) = (Jarak titik A-C) – (Jarak titik a-c)

Pengukuran 2 (mm) = (Jarak titik B-D) – (Jarak titik b-d)

$$\text{Zona daya hambat (mm)} = \frac{\text{Pengukuran 1} + \text{Pengukuran 2}}{2}$$

Figure 1. Illustration of Inhibition Zone Diameter Maesurement

The data obtained from the research results were analyzed using the normality test (Shapiro-Wilk) and homogeneity test (Levene). If the data is distributed normally and homogeneously ($p>0.05$), a parametric test is carried out, namely one-way ANOVA, to determine whether there is an effect of beetroot extract in inhibiting the growth of Enterococcus faaecais bacteria, then proceed with the Post-Hoc Bonferroni method test to determine the differences. between treatments. If the data is not normally and homogeneously distributed ($p<0.05$), then the Kruskal-Wallis nonparametric test is carried out followed by the Mann-Whitney test.

3. RESULTS

Test for measuring the inhibition zone of beetroot extract against Enterococcus faecalis using the disk diffusion method at concentrations of 10,000µg/ml, 12,500µg/ml, and 15,000µg/ml with a positive control of 2% Chlorhexidine Gluconate and a negative control of distilled water. The inhibition zone which is visible as a clear area around the disc is measured manually with a caliper in millimeters (mm), and the diameter is then calculated using the normalization formula for the width of the inhibition area. This research was carried out three times as seen in Figure 2 and Table 1

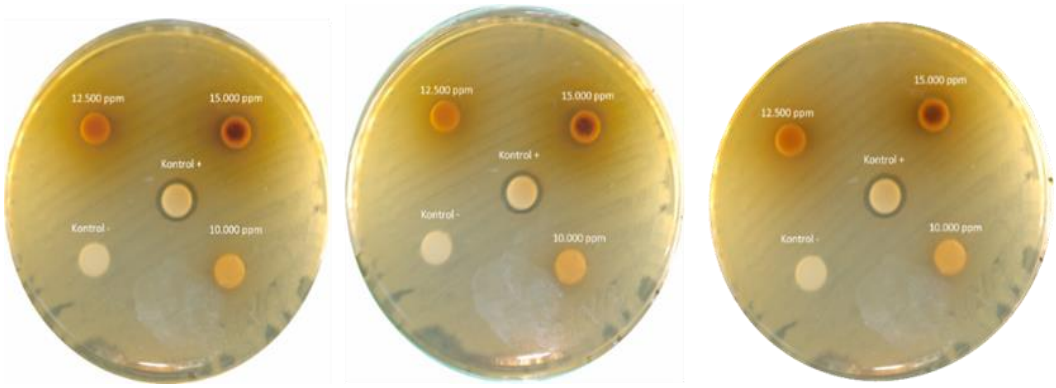

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Figure 2. Inhibition Zone Test Results Using Disc Diffusion Method After 24 Hours Incubation, Repetition I (Left), Repetition II (Middle), Repetition III (Right)

Tabel 1. Inhibition Zone Measurement Results After 24 Hours Incubation

Treatment Group	Average ± SD
Beetroot Extract 10,000 µg/ml	0,00±0,00 mm
Beetroot Extract 12,500 µg/ml	1,77±0.06 mm
Beetroot Extract 15,000 µg/ml	2,33±0,06 mm
Chlorhexidine Gluconate (CHX) 2%	2,53±0,06 mm
Aquades	0,00±0,00 mm

4. DISCUSSION

Measurement of the inhibition zone at four types of beetroot extract concentrations showed that not all concentrations could inhibit the growth of Enterococcus faecalis bacteria. The average diameter of the inhibition zone varies and increases with increasing concentration levels of beetroot extract. Table 5 shows that a beetroot concentration of 15,000 µg/ml produces an inhibitory zone against Enterococcus faecalis bacteria with the largest average measuring 2.33 ± 0.06 mm, followed by a concentration of 10,000 µg/ml at 0.00 ±

0.00 and 12,500 µg/ml of 1.77 ± 0.06 mm. The positive control, Chlorhexidine Gluconate 2%, is the sample group that has the largest average inhibition zone, namely 2.53 ± 0.06 and the negative control distilled water has an average inhibition zone of 0.00 ± 0.00, which means there is no antibacterial effectiveness. against Enterococcus faecalis.

In this study, beetroot extract showed antibacterial activity. The results of the research show that beetroot extract has an antibacterial effect against the bacteria Enterococcus faecalis ATCC 29212. The results of this test are in line with Yasmin, et al who show that beetroot extract has an antibacterial effect against Gram-positive bacteria, namely Streptococcus mutans[11], and beetroot extract is able to create an inhibitory zone. around the paper disc, in accordance with the sensitivity of the bacteria in this study determined by inhibition zone measurements.

The strength of the inhibitory zone was categorized by Ahn based on the size of the resulting inhibitory zone, namely weak (<5mm), medium (5-10mm), strong (10-20mm), and very strong (>20mm).[53] The largest average diameter of the inhibitory zone of the extract beetroot (Beta vulgaris L.) is at a concentration of 15,000 µg/ml, followed by a concentration of 12,500 µg/ml which has a smaller diameter of the inhibition zone, and a concentration of 10,000 µg/ml indicates no inhibition zone is formed, this indicates that the concentration is higher. The height will be directly proportional to the inhibition zone produced, the results obtained are in accordance with the concentration dependent theory.[61] This statement is supported by research by Saani, et al who examined the ethanol extract of beetroot against Gram positive bacteria which showed that the inhibition zone increased when the extract concentration increased.[58] This research is also in line with Razak, et al who showed that the smaller the concentration of beetroot extract will result in a smaller zone of inhibition as well.[64]

Several other studies show that the ingredients in beetroot are able to inhibit the growth or kill anaerobic bacteria other than Enterococcus faecalis, one of which is proven by research conducted by Hutasuhut, et al. that beetroot extract is able to inhibit the growth of Escherichia Coli bacteria.[65] The antibacterial activity is in the form of inhibiting and killing Enterococcus faecalis can be caused by the role of bioactive compounds contained in beetroot extract.[59,60]

One of the ingredients in beets that is very effective in inhibiting and killing Enterococcus faecalis bacteria is phenol. Phenol is able to damage cell membranes, inactivate enzymes, and denature proteins, so that cell walls are damaged due to permeability, causing leakage of nutrients from bacterial cells and bacterial growth will be hampered or even die. Phenolic compounds are bacteriostatic depending on the concentration used, increasing the concentration of beetroot extract is directly proportional to increasing levels of phenol contained in beetroot extract, thereby increasing its inhibitory power against Enterococcus faecalis.[56] This is proven by research conducted by Ramadhinta, et al. that the content The phenols in orange juice can inhibit and even kill Enterococcus faecalis bacteria.[57]

It is hoped that beetroot extract can be developed as an alternative antibacterial agent in dentistry. According to a number of previous studies, beetroot extract has very little toxicity. A dose of 500 mg/kgbb of beetroot ethanol extract is not harmful to Wistar rats, according to research by Gamal et al.[62] Due to the high levels of phenols and flavonoids in beetroot, research by Kim et al. in vivo studies show that beet ethanol extract can have preventive and therapeutic effects on healing liver damage.[63]

5. CONCLUSION

Beetroot extract (Beta vulgaris L.) has an antibacterial effect against Enterococcus faecalis bacteria starting from a concentration of 12,500 µg/ml.

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