"Research Article"

The Antibacterial Effect of Red Dragon Fruit Peel Extract (Hylocereus Polyrhizus) Against The Growth of Bacterium Enterococcus Faecalis

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Abstract

Enterococcus faecalis is the main cause of periradicular infections after root canal treatment. It is currently being encouraged by using natural ingredients as an alternative tooth root canal irrigation substance, one of which is the peel of red dragon fruit (Hylocereus polyrhizus) containing flavonoids, tannins, and alkaloids. To identify the antibacterial effects of the peel of red dragon fruit extract (Hylocereus polyrhizus) on the growth of the bacterium Enterococcus faecalis. This experimental laboratory research in vitro using the bacterium Enterococcus faecalis ATCC 2921. The method used was ditch-diffusion on Mueller Hinton media to continue measuring a radical zone using a sliding caliper. The peel of red dragon fruit extract (Hylocereus polyrhizus) consists of several concentrations: 20%, 40%, 60%, 80%, and 100% based on V1N1 = V2N2. Data were tested statistically using the One way-ANOVA test, starting with a normality test using the Shapiro-Wilk test, then a homogeneity test, and continued with a post hoc test using the Least Significant Difference (LSD) to identify whether the results showed a significant difference in the antibacterial power of dragon fruit peel extract against Enterococcus faecalis. All tested concentrations were antibacterial against Enterococcus faecalis bacteria growth. The highest concentration of antibacterial against was 100% (p <0.05). Peel of red dragon fruit extract (Hylocereus polyrhizus) had an antibacterial effect on the growth of Enterococcus faecalis bacteria.

Keywords: Peel of red dragon fruit extract (Hylocereus polyrhizus), Enterococcus faecalis, antibacterial.

INTRODUCTION

Root canal treatment includes three stages: preparation, sterilization, and filling. This preparation stage is very important due to its ability to remove 80% of necrotic tissue biomechanically, then the remaining 20% of necrotic tissue can be removed by root canal irrigation. This root canal irrigation aims to kill the remaining bacteria in the root canal and dentin tubules that cannot be reached during the chemomechanical preparation of the root canal.

Cases of root canal treatment failure reach a high percentage of up to 77%. Statistical analysis of the causes that could lead to endodontic treatment failure two years after completion showed that of 1229 cases treated endodontically, 1.5% were successful without complaints, and the remaining 8.5% experienced failure.

Previous research revealed that 63% of root canal treatment failures that experienced reinfection were caused by Enterococcus faecalis. Enterococcus faecalis is a gram-positive bacterium that has a coccus shape and is a facultative anaerobic bacterium that can survive in water under conditions of starvation for long periods and in areas where there is a lot of water, such as dentinal tubules and body fluids. Enterococcus faecalis is the main cause of periradicular bacteria after root canal treatment.

Root canal irrigation materials can eliminate microorganisms in the root canal. There are several types of root canal irrigation materials, such as conventional disinfection materials, namely phenolic compounds, formaldehyde, and halogens, which have been abandoned as they are irritating, as well as NaOCl (sodium hypochlorite), EDTA (Ethylene Diamine...
Tetraacetic Acid), chlorhexidine and Ca(OH)2 (Calcium Hydroxide). 5% NaOCl root canal irrigation material can dissolve organic debris such as pulp tissue and collagen, dissolve the smear layer that cannot be reached by instruments, and, as an antimicrobial agent, can eliminate endotoxins and eradicate bacteria in the root canal, root canal walls and dentinal tubules. However, the 5% NaOCl solution has several disadvantages. It has an unpleasant odor and taste, is unable to remove the entire smear layer, can change the structure of the dentin and can interfere with pulp regeneration, is toxic, and can cause a feeling of discomfort when it enters the periapical tissue, bleeding in the root canal, hemorrhage, edema, and irritation. The toxic effects of 5% NaOCl can be minimized by using safe alternative materials for root canal irrigation.

The results of research conducted by Nurliyana et al. showed that the total phenol content of red dragon fruit peel was higher than the ethanol extract of red dragon fruit flesh. Red dragon fruit peel contains one group of phenolic compounds, namely flavonoids, which are beneficial for the body. It is known that the n-hexane, chloroform, and ethanol extracts of red dragon fruit peel have antibacterial activity against gram-positive and gram-negative bacteria. Ethanol extract from red dragon fruit peel can inhibit the growth of several bacteria, including Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, Yersinia enterocolitica, and Klebsiella pneumonia based on the results of research conducted by Nurmahani in 2012. Several active compounds in red dragon fruit peel apart from flavonoids, which are thought to have antibacterial activity based on the results of phytochemical screening, are alkaloids and terpenoids. The role of phenol or polyphenol compounds in dragon fruit peel can kill microorganisms and inhibit the production of bacterial toxins, causing these components to change the permeability of microorganism cells and allow the loss of macromolecules in cells. Phenolic compounds can also interact with membrane proteins, causing structural and functional changes in bacteria. The antibacterial activity of phenol compound can inhibit several types of bacteria, especially gram-positive bacteria.

Based on the description above, this research aims to identify the content of red dragon fruit peel (Hylocereus polyrhizus), which has antibacterial properties against Enterococcus faecalis bacteria.

**MATERIALS AND METHOD**

The research design employed in this study was purely experimental laboratory in vitro with a posttest-only with a control group design. The parameters of this research were the radical zones formed from the materials tested. The sample groups in this study were pure cultures of Enterococcus faecalis ATCC 29212 and red dragon fruit peel extract (Hylocereus polyrhizus) concentrations of 20%, 40%, 60%, 80%, and 100%, as well as sterile distilled water as a negative control and 5% NaOCl. In each treatment group sample, the treatment was replicated 5 times.

Prior to the antibacterial power test, the tools and materials were sterilized. The tools were washed thoroughly and dried, including test tubes, flasks, buncher funnels, Petri dishes, and other tools made of glass covered at the mouth with cotton, then wrapped in newspaper, then sterilized using an autoclave at 121°C for 15 minutes. The tools sterilized were Elenmeyer tubes, measuring cups, Petri dishes, perforators, MH media, BHI, and test tubes. Hose needles were sterilized using exposure directly over a flame.

The red dragon fruit peel was washed, then cut into small pieces and dried in a drying cabinet at a temperature of 45°C for 48 hours. The dried red dragon fruit peel was then ground using a blender and sieved with a 50 mesh sieve, and fine simplicia powder was obtained. The simplicia was then soaked in a 70% ethanol solution with...
a ratio of solvent (b/v) and solvent concentration in a closed glass jar for 24 hours. It was stirred periodically for 30 minutes using a magnetic stirrer and then left for 24 hours at room temperature. After 24 hours, it was stirred again, left for another 24 hours, and filtered using a Buchner funnel to separate the filtrate from the residue. The filtrate was evaporated using a vacuum rotary evaporator to evaporate 70% ethanol for one hour at 60-70°C, producing a thick extract. The thick extract was put into a porcelain cup, and the remaining solvent from the thick extract was evaporated using a water bath at 50°C. The thick extract dosage was dissolved in sterile distilled water to obtain a concentration of 20%, 40%, 60%, 80%, and 100%.

MHA that has been sterilized was dissolved in 38 grams in 1 liter of distilled water. It was sterilized using an autoclave at 121°C for 25 minutes and left until the temperature dropped to 40°C. It was then poured into a sterile petri dish containing 15-20 mL and left until it solidified.

Bacterial suspensions were prepared using Brown III standards of 10^8 CFU/ml. The suspension was made by taking several tubes of Enterococcus faecalis bacteria using a sterile tube, then putting it in 1 ml of NaCl and shaking it until homogeneous. It was incubated for 3-5 hours at 37°C. The NaCl solution with bacteria was added to 9 ml of BHI liquid media with a standard concentration of 10^8 CFU/ml.

This study utilized the well diffusion method, namely creating wells with a 6 mm diameter perforator on the surface of the agar media, which had been inoculated with bacteria. Furthermore, red dragon fruit peel extract was dripped with concentrations of 20%, 40%, 60%, 80%, and 100%. 5% NaOCl as a positive control, and sterile distilled water as a negative control using a micropipette. The petri dish containing the test bacteria and solution was put into the anaerobic jar, followed by an incubation process for 24 hours at 37°C. Bacterial growth can be observed, and the radical zone's diameter around the hole can be measured.

Radical zone measurements can be read after being incubated for 24 hours at 37°C in an anaerobic atmosphere and measured using a sliding caliper. The radical zone around the well indicates the bacteria's sensitivity to the antibacterial agent used as the test material.

The research data was calculated manually, and then the data was tested statistically using the One way-Anova test, starting with a normality test using the Shapiro-Wilk test, then a homogeneity test, and continued with a post hoc test using the Least Significant Difference (LSD) to identify whether the results showed a significant difference in the antibacterial power of dragon fruit peel extract against Enterococcus faecalis.

**RESULT**

<table>
<thead>
<tr>
<th>Test solution</th>
<th>Repetition</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>20%</td>
<td>6.97</td>
<td>6.67</td>
</tr>
<tr>
<td>40%</td>
<td>7.57</td>
<td>7.9</td>
</tr>
<tr>
<td>60%</td>
<td>9.67</td>
<td>8.67</td>
</tr>
<tr>
<td>80%</td>
<td>11.97</td>
<td>12.57</td>
</tr>
<tr>
<td>100%</td>
<td>15.45</td>
<td>14.93</td>
</tr>
<tr>
<td>Sodium Hypochlorite 5%</td>
<td>15.83</td>
<td>14.5</td>
</tr>
<tr>
<td>Aquades</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1. Radical zone measurement results
The results in Table 1 show that no radical zones were formed in the aquades negative control. The well of the sodium hypochlorite, as a positive control, showed an average radical zone of 14.592 mm. Various concentrations of red dragon fruit peel extract (Hylocereus polyrhizus) demonstrated that the higher the red dragon fruit peel extract concentration (Hylocereus polyrhizus) is, the greater the radical zone will be formed. A concentration of 20% shows a mean of 7.372 mm, a concentration of 40% shows a mean of 8.396 mm, a 60% concentration shows a mean of 9.4 mm, an 80% concentration shows a mean of 12.458 mm, and 100% concentration shows a mean of 14.98 mm.

Research data in the form of the radical zone size for each group was then analyzed using the SPSS application. The first test is the data distributor.

**Table 2. Shapiro-Wilk Normality Test**

<table>
<thead>
<tr>
<th>Group</th>
<th>Significance</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>20%</td>
<td>.619</td>
<td>p&gt;0,05</td>
</tr>
<tr>
<td>40%</td>
<td>.787</td>
<td>p&gt;0,05</td>
</tr>
<tr>
<td>60%</td>
<td>.968</td>
<td>p&gt;0,05</td>
</tr>
<tr>
<td>80%</td>
<td>.403</td>
<td>p&gt;0,05</td>
</tr>
<tr>
<td>100%</td>
<td>.985</td>
<td>p&gt;0,05</td>
</tr>
<tr>
<td>Sodium Hypochlorite 5%</td>
<td>.842</td>
<td>p&gt;0,05</td>
</tr>
</tbody>
</table>

The normality test results of the group using the Shapiro-Wilk test (number of data ≤ 50) demonstrated normal data distribution with a significance value of p>0.05. Data testing continued with the homogeneity test to identify whether the data groups had the same variance.

**Table 3. Homogeneity Test**

<table>
<thead>
<tr>
<th>Radical zone of bacterial growth Enterococcus faecalis</th>
<th>Sig.</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,127</td>
<td>.082</td>
<td>P&gt;0,05</td>
</tr>
</tbody>
</table>

The homogeneity test results showed a significance value of p>0.05, indicating the data had the same variance. Therefore, the hypothesis testing was then carried out using the One-Way Anova parametric analysis test.

**Table 4. One Way Anova parametric test**

<table>
<thead>
<tr>
<th>Variable</th>
<th>sig</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>A radical zone</td>
<td>0,000</td>
<td>P&lt;0,05</td>
</tr>
</tbody>
</table>

Based on Table 4, the significance value of p=0.000 is obtained, where the p-value is <0.05, indicating that H0 is rejected and H1 is accepted. It demonstrated that the red dragon fruit peel extract (Hylocereus polyrhizus) had antibacterial power in inhibiting the growth of Enterococcus faecalis bacteria. Testing continued with post hoc analysis with the Multiple Comparison LSD (Least Significant Difference) test to identify which groups had meaningful and significant mean differences.

A significant mean difference can be seen when the significance p-value is <0.05. Post hoc testing showed that the p-value was <0.05, indicating that the groups had a significant mean difference as an antibacterial power.

**DISCUSSION**

Based on research results, red dragon fruit (Hylocereus polyrhizus) peel extract had antibacterial power against the growth of Enterococcus faecalis bacteria by forming a radical zone. The results of this research are supported by previous research conducted by Suhartati R et al. (2017), revealing that ethanol extract of red dragon fruit peel (Hylocereus polyrhizus) concentrations of 20%, 40%, 60%, 80%, 100% could inhibit the growth of Streptococcus pyogenes bacteria. The higher the concentration of the extract is used, the greater the power of its antibacterial will be.

According to research on the antibacterial activity of the n-hexane fraction of red dragon fruit peel (Hylocereus polyrhizus) conducted by Amalia Sri et al. (2014), it showed that
dragon fruit peel contains several compounds with antibacterial power. Several compounds in the n-hexane fraction are suspected of having antibacterial activity based on phytochemical screening, including alkaloids, steroids, and triterpenoids. Steroid and triterpenoid compounds consist of several ingredients such as β-amyrin (15.87%), α-amyrin (13.90%), (12.2%), γ-sitosterol (9.35%), octadecane (6.27%), tetracosanol (5.19%), cytostenone (4.65%), and campesterol (4.16%) [21]. Steroids can interact with cell phospholipid membranes, which are impermeable to lipophilic compounds, causing membrane integrity to decrease and cell morphology to change, ultimately causing cell brittleness and lysis [22].

Triterpenoids can react with transmembrane proteins in bacterial cell membranes and then form strong polymer bonds, causing porins to be damaged. Porins are the entry and exit points for nutrients for bacteria. A damaged principle can cause bacteria to lack nutrition, leading to their growth being hampered and death [22].

Based on the discussion above, it showed that the mechanism of the alkaloid, flavonoid, tannin, steroid, and triterpenoid compounds contained in red dragon fruit (Hylocereus polyrhizus) peel extract had antibacterial power against the growth of Enterococcus faecalis. Red dragon fruit peel extract with a concentration of 100% had the highest antibacterial power compared to concentrations of 20%, 40%, 60%, and 80% and had a radical zone diameter larger than the radical zone produced by 5% NaOCl. Thus, the red dragon fruit peel extract can be used as an alternative material for root canal irrigation. 

CONCLUSION

All concentrations of red dragon fruit (Hylocereus polyrhizus) peel extract tested in this study had antibacterial power against the growth of Enterococcus faecalis bacteria. 100% concentration of red dragon fruit (Hylocereus polyrhizus) peel extract had the highest antibacterial power that functions as an antibacterial and works by bonding through hydrogen to form a complex compound with pretin. Once the bond is formed, the protein will denature, causing bacterial cell metabolism to be disrupted and lysis and death of the bacterial cell will occur [24].

Alkaloids are bioactive substances that function as antibacterials and work by inhibiting and disrupting peptidoglycan when it is arranged in bacterial cells, causing the cell membrane to be damaged and unable to return to normal [22]. Alkaloid compounds consist of base groups containing nitrogen which will react with amino acid compounds found in bacterial cell walls and bacterial DNA. This reaction can cause changes in the structure and arrangement of amino acids in the bacterial cell wall, leading to changes in the genetic balance of the DNA chain, resulting in lysis and even death of the bacterial cell (Gunawan, 2009).

Tannin is a bioactive substance that functions as an antibacterial and works by bonding through hydrogen to form a complex compound with pretin. Once the bond is formed, the protein will denature, causing bacterial cell metabolism to be disrupted and lysis and death of the bacterial cell will occur [24].

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identified from the average radical zone formed.

It is important to develop new antibacterial agents by exploring natural ingredients - including red dragon fruit - since bacterial resistance caused by inappropriate and inadequate use of antibiotics increasing lately.

REFERENCES


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