Effectiveness of Mixed Clove Flower Extract (Syzygium Aromaticum) And Sweet Wood (Cinnamon Burmanni) on the Growth of Enterococcus Faecalis

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Background

Failed root canal treatment can cause problems such as recurring pain and leave persistent bacteria in the root canal. One of the bacteria that often causes root canal treatment failure is Enterococcus faecalis. The use of a root canal irrigation agent such as sodium hypochlorite (NaOCl) can help inhibit the growth of bacteria in the root canal. The use of materials derived from nature can be used as an alternative material for root canal irrigation because they inhibit growth and kill bacteria and have fewer side effects than chemicals. Several studies have tried to prove the use of ingredients of natural origin in the form of a mixture and the results obtained are higher when compared to single use. Natural ingredients that are often used as herbal medicine include cloves and cinnamon. **Objective.** To determine the effectiveness of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) on the growth of Enterococcus faecalis. Methods. Using experimental laboratory methods, with a research design in the form of Post Test Only Control Design, sampling by random sampling using 5 treatments and 5 repetitions. Statistical test using One Way Anova. **Results.** This study showed the diameter of the inhibition zone of Enterococcus faecalis bacteria in a mixture of clove flower (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) extract at 5% concentration of 10.84 ± 0.73, 10% concentration of 15.00 ± 0.80 mm. The concentration of 15% was 16.32 ± 1.04 mm and based on statistical tests obtained a significant value of P <0.01. **Conclusion.** The alternative hypothesis of this study is accepted and this study shows that there is an effectiveness of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) concentrations of 5%, 10%, and 15% in inhibiting the growth of Enterococcus faecalis.

Keywords: *Enterococcus Faecalis, Extract Clove Flowers (Syzygium Aromaticum), Extract Cinnamon (Cinnamon Burmanni)*

Introduction

Root canal treatment is a treatment option that can be used to treat pulp tissue infections. Failed root canal treatment can cause problems such as recurring pain and leave persistent bacteria in the root canal. From various research results, it was found that the microorganism that often causes root canal treatment failure is the bacterium Enterococcus faecalis. Enterococcus faecalis is a facultativeanaerobic gram-positive bacteria and is one of the most resistant microorganisms among the root canal microflora.

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The main goal of pulp and root canal treatment is to remove microbes from the pulp and periapical tissues. Root canal treatment is influenced by proper instrumentation, disinfection, and obturation of the root canals. During root canal preparation, irrigation is very important for several reasons, namely for cleaning the root canals, lubricating files during preparation, cleaning residual debris, removing dentin tissue or smear layers, and removing bacteria without affecting the periapical tissue. The ideal irrigation solution must have two main qualities, namely having an antibacterial effect and low toxicity to periapical tissue. [4],[5]

The use of a root canal irrigation agent such as sodium hypochlorite (NaOCl) can help inhibit the growth of bacteria in the root canal. NaOCl is inadequate in eliminating persistent bacteria. The concentrations commonly used range from 0.5% to 5.25%. The use of materials derived from nature can be used as alternative materials for root canal irrigation because they inhibit growth and kill bacteria. Traditional medicines are considered to have less side effects than drugs derived from chemicals. Indonesia is a country that is rich in biodiversity. Of the total 28,000 species of medicinal plants in Indonesia, 1,845 have been identified as having medicinal properties.[6]

Several studies have tried to prove the use of plants in the form of mixtures or combinations of several types of plants as reported by Miksusanti et al. (2012) with mangosteen rind extract and secang wood against antioxidant activity, Wajdi et al. (2017) with a mixture of Moringa seed extract and cherry leaves against Pseudomonas aeuroginosa and Bacillus subtilis, and the results obtained were higher when compared to single use. Therefore, research related to extract mixtures is important to do in order to increase the biological activity of several types of plants whose activity is known in the form of single use.[7]

Two types of plants that are known to have a single biological activity are cloves and cinnamon. The stems, leaves, and flowers of the clove plant have many benefits. Clove flower is used traditionally as an antimicrobial. Extracts from clove flowers have biological activities, such as antibacterial, antifungal, insecticidal, topical analgesics, sedatives and antioxidants. The content of antibacterial compounds in clove flowers, namely flavonoids, tannins, alkaloids, and eugenol.[8]

Cinnamon is known to be used as antibacterial, antifungal, anti-inflammatory, analgesic, antioxidant, inhibits the formation of dental plaque and periodontal disease, as well as other activities. Chemical compounds that are thought to act as antibacterials in cinnamon are known to be used as antibacterial, antifungal, anti-inflammatory, analgesic, antioxidant, inhibiting the formation of dental plaque and periodontal disease, as well as other activities. Chemical compounds that are thought to have an antibacterial role in cinnamon are essential oils of about 0.5–2% (such as eugenol, safrol, cinnamaldehyde and linalool), polysaccharides around 10% (such as diterpenes and coumarin), 4–10% phenolic components (such as tannins) and flavonoids.[9]

Based on the description above, the researcher is interested in conducting research on the effectiveness of a mixture of clove flower extract and cinnamon on the growth of the bacteria Enterococcus faecalis. “The researchers hope that these clove and cinnamon plants can complement each other so that they can have a strong effect.

Methods

The research design used was true experimental research design with post test only control group design and sampling with random sampling using 5 treatments and 5 repetitions. Statistical test using One Way Anova. Testing the ability of the zone of inhibition of a mixture of clove flower extract and cinnamon using the agar diffusion method.

This research was conducted in the Microbiology Laboratory of the Hasanuddin University Medical

Faculty. The sample was Enterococcus faecalis bacteria from the pure bacterial stock of the Hasanuddin University Microbiology Laboratory. The independent variable (independent) studied was a mixture of clove flower extract and cinnamon made by maceration method using 96% ethanol solvent and made concentrations of 5%, 10% and 15% with positive control 5.25% NaOCl and sterile Aquades as negative control.

The materials used in this study were clove flowers (Syzygium aromaticum) and cinnamon (Cinnamon burmanni). Enterococcus faecalis bacteria, 96% ethanol, 5.25% NaOCl, DMSO (Dimethyl sulfoxide), Mueller Hinton Agar (MHA), and paperdisk. The tools used in this research are autoclave, incubator, analytical scale, petri dish, sterilizer / oven, flatware, round loop, tweezers, digital calipers, erlenmeyer tube, vial bottle, dropper, filter paper jar.

Result

The results showed that a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a concentration of 5%, 10%, 15% had antibacterial power against Enterococcus faecalis as indicated by the presence of a clear zone formed around the paper disk.

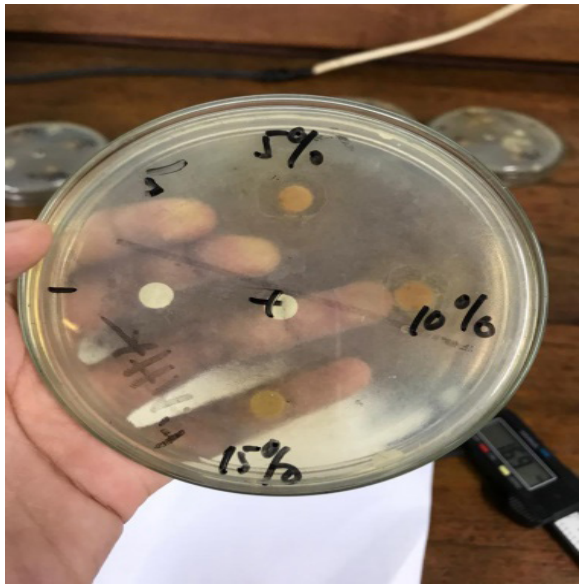

Figure 1. Inhibition zone formed on MHA media

The inhibition zone produced by each treatment has a different diameter. Observations are made by measuring the horizontal diameter and vertical diameter of the formed drag zone and then enter it into the formula to find the mean of the drag zone and then enter it in the worktable.

Table 1. The average diameter of the zone of inhibition of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a concentration of 5%, 10%, 15%, on the growth of Enterococcus faecalis bacteria and the results of data normality test.

Type of Solution		Mean ± SD	p-value Shapiro Wilk
Mixed solution of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni)	Concentration 5%	10,84± 0,73	0,872
	Concentration 10%	15,00 ± 0,80	0,731
	Concentration 15%	16,32 ± 1,04	0,529
Positive Control	NaOCl 5,25%	21,74± 3,26	0,979
Negative Control	Aquades	0,00±0,00	-

Note: Normality Test; Shapiro-Wilk test: p> 0.05, normal data distribution

* Anova One-way test: p <0.01: significant

Discussion

This research was conducted at the Microbiology Laboratory of the Faculty of Medicine, Hassanuddin University for two days, which was conducted in January 2021. This study was conducted with the aim of determining the effectiveness of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) on the growth of Enterococcus faecalis. In this study using clove flower extract and cinnamon extract and then the extraction process was carried out using the maceration method. After obtaining the thick extract, then diluting it to make a concentration of 5%, 10% and 15%.

The effectiveness of the inhibition was carried out using the inhibition zone observation method by looking at the clear zone around the paper disk which was given a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) 5%, 10%, 1 5%, NaOCl, respectively. 5.25% as a positive control, and Aquades as a negative control that was incubated in the incubator for 1 x 24 hours.

The visible zone of inhibition indicates an inhibition of Enterococcus faecalis bacteria on agar medium. The agar medium used was Muller Hinton Agar (MHA) which contained the bacteria Enterococcus faecalis. Muller Hinton Agar (MHA) was chosen as the agar medium used in the study because it is the most selective medium for bacterial growth and is more optimal when incubation is incubated.

The results of this study indicated that a mixed solution of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) could inhibit the growth of Enterococcus faecalis at each concentration. The biggest zone of bacterial inhibition is in a solution of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a concentration of 15% of 16.32 ± 1.04 mm, so it can be categorized as moderate in inhibiting Enterococcus faecalis bacteria, while the power zone The smallest inhibition was a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a 5% concentration of 10.84 ± 0.73 mm, and could be categorized as weak in inhibiting Enterococcus faecalis bacteria.

This result is supported by research conducted by Rastina et al. (2015) said that the ability of an antimicrobial to inhibit microorganisms depends on the concentration of the antimicrobial material and the type of antimicrobial material produced. The greater the concentration of an antimicrobial, the larger the clear zone is formed. This is because the higher the concentration of antimicrobial ingredients, the more active substances contained in it so that the effectiveness in inhibiting bacteria will increase and produce a wider clear zone. Conversely, at low concentrations, the less antimicrobial substances contained in an antimicrobial agent will reduce their activity.[10]

Lorain (2005) explains that the greater the antimicrobial concentration, the faster diffusion occurs, so that the antibacterial power will be greater and the resulting inhibition zone diameter will be wider. This result is supported by the statement of Prawata and Dewi (2008), that the effectiveness of an antibacterial substance is influenced by the concentration of the substance. Increasing the concentration of substances causes an increase in the content of active compounds that function as antibacterials, so that their ability to kill bacteria is also greater. However, the effectiveness of the antibacterial action of a substance can be influenced by several factors, including the antibacterial concentration, the number of bacteria, bacterial species, organic matter, temperature, and environmental pH. This is also supported by the statement of Jenie and Kuswanto (1994) which states that the effectiveness of an antibacterial substance in inhibiting growth depends on the nature of the tested bacteria, concentration and length of contact time. [11],[12],[13]

This study aims to determine that a mixture of clove flower extract and cinnamon can inhibit the growth of the bacteria Enterococcus faecalis. Which is the effectiveness of a mixture of clove flower extracts and cinnamon against the inhibition of Enterococcus faecalis bacteria as an antibacterial agent. This is in line with research conducted by Huda et al. (2018) which states that the content of antibacterial compounds in clove flowers, namely flavonoids, tannins, alkaloids, and euganol. Meanwhile, according to Muslim, et al (2018) stated that Cinnamon extract contains cinnamaldehyde compounds as antibacterials. Which is the mechanism of the cinnamaldehyde compound in cinnamon extract is to inhibit energy metabolism in bacteria. This is evidenced by the synthetic inhibition of L. monocytogenes bacterial cell walls and inhibiting the biosynthetic enzymes used for the formation of energy.[14],[15]

Based on the research of Suhendar et al (2019) regarding the antibacterial activity of clove flower extract (Syzygium aromaticum) against Streptococcus mutans bacteria, the results of the qualitative phytochemical test showed that the methanol extract of clove flowers contained alkaloid, flavonoids, terpenoids and phenolic compounds. The steroid compound was not identified in this test. Based on the results of the antibacterial activity test, clove flower extract produced its inhibition zone. The inhibition zone indicates that the clove flower extract has the potential to be antibacterial. Clove flower extract has antibacterial activity by inhibiting S mutans bacteria, inhibition zone diameter is 37 mm, and positive control is 28 mm. The formation of an inhibitory zone around the well / disc indicates the inhibitory activity of clove flower extract against S. mutans bacteria.[8]

The formation of bubbles after adding one drop of 0.1 HCl indicates that the sample contains saponins. The formation of a white precipitate after the addition of 10% gelatin proves that the sample contains tannins. The green-black color formed after the addition of 1% FeCl3 solution indicates the presence of polyphenols in the cinnamon extract. Meanwhile, the brown color formed after Mg and 1 ml of HCl were added, indicating that the sample contains flavonoids. Quinone was detected in the cinnamon extract due to the formation of a red color due to the addition of 1% NaOH. The formation of a red color after the addition of Carr Price's reagent indicates that the sample contains triterpenoids. From this study, it can be concluded that Cinnamon burmannii has antibacterial activity in the form of the ability to inhibit the growth of Enterococcus faecalis.[9]

Conclusion

Based on the results of the research conducted, it can be concluded:

1. There is the effectiveness of a mixture of clove flower extracts (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a concentration of 5% with an average inhibition zone of 10.84 ± 0.73 mm in reducing Enterococcus faecalis bacteria.

2. There is an effectiveness of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a concentration of 10% with an average inhibition zone of 15.00 ± 0.80 mm in inhibiting Enterococcus faecalis bacteria.

3. There is an effectiveness of a mixture of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni) with a concentration of 15% with an average inhibition zone of 16.32 ± 1.04 mm in inhibiting Enterococcus faecalis bacteria.

4. The most effective treatment in inhibiting the growth of Enterococcus faecalis bacteria is a mixture of 15% concentration of clove flower extract (Syzygium aromaticum) and cinnamon (Cinnamon burmanni).

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Ethical Considerations: Ethical clearance was obtained from Universitas Muslim Indonesia, Makassar; with number" 009/A.1/KEPK-UMI/I/2021. Just before the interview, written (or

thumb impression) consent was obtained from each participant in Universitas Muslim Indonesia, Makassar guidelines.

Conflicts of Interest: The authors alone are responsible for the views expressed in this article and they do not necessarily represent the views, decisions, or policies of the institutions with which they are affiliated.

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