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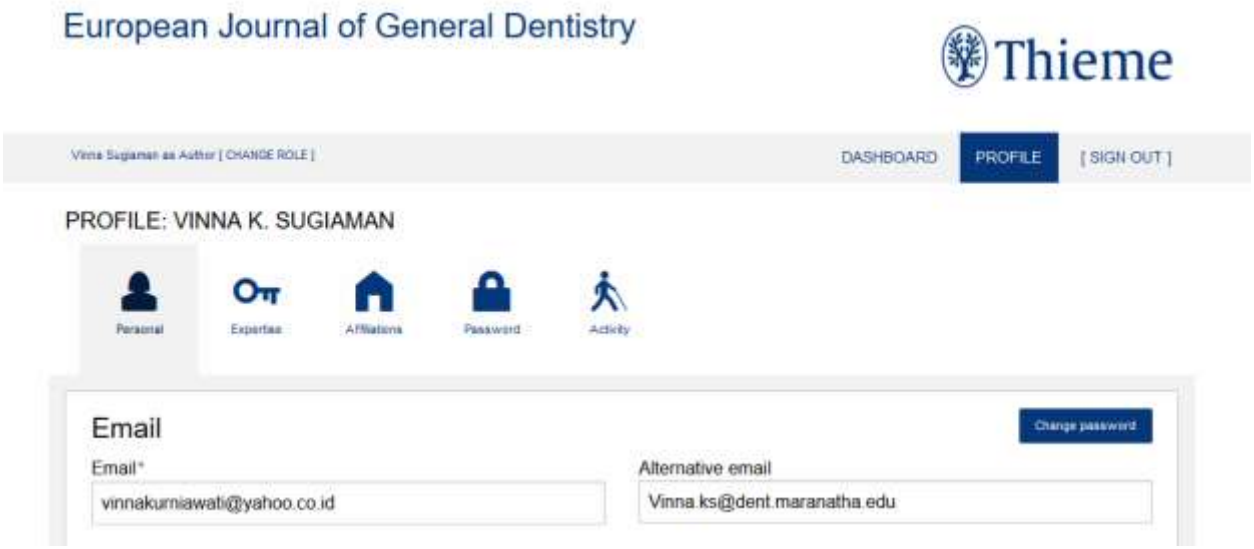

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Authors: Vinna Kurniawati Sugiaman (Corresponding Author), Pitri Ayu Puspita Sari (Co-author), Rudy Djuanda (Co-author)
Date submitted: 2024-03-31

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Effect of Saga Leaf Extract (*Abrus precatorius Linn*) in Inhibiting *Enterococcus faecalis* Bacteria Growth as an Alternative Root Canal Irrigation Material

ABSTRACT

Irreversible pulpitis and pulp necrosis are caused by bacteria. *Enterococcus faecalis* is a bacterium that often causes failure in root canal treatment. The failure of root canal treatment can be prevented with appropriate and non-toxic irrigating solutions, such as saga leaves. Saga (*Abrus precatorius Linn*) is a plant that contains antibacterial compounds in the form of phenolics, tannins, saponins, steroids, and flavonoids, especially in its leaves. Researchers are interested in examining the antibacterial effect of saga leaf extract on *Enterococcus faecalis* bacteria, which causes root canal treatment failure. This research was conducted using a laboratory experimental method with saga leaf extract at 3.125%, 6.25%, 12.5%, 25%, 50%, and 100% concentrations. 2% Chlorhexidine acts as the positive control, and 100% DMSO as the negative control. The diameter of the inhibition zones were measured using the well-diffusion test method. The largest inhibition zone diameter observed in this study was 9.46 mm at 100% concentration; however, it was not bigger than the positive control, which was measured at 16.55 mm. The research data were analyzed based on the classification of Davis and Stout inhibition zones. This study concludes that saga leaf extract has an antibacterial effect on the growth of Enterococcus faecalis bacteria so it is hoped can be an alternative material for root canal irrigation.

***Keywords**: Enterococcus faecalis, Abrus precatorius Linn, root canal treatment, well diffusion, antibacterial*

INTRODUCTION

Pulp necrosis and irreversible pulpitis are the most common cases found in dentistry. They can be prevented and treated with root canal treatment.[1,2] Root canal treatment is a dental procedure that aims to maintain or improve the condition of infected tooth in order to be accepted biologically by the tissues. Successful root canal treatment depends on the ability to eliminate microorganisms from the root canal system and the re-infection prevention.[3,4]

The most common root canal microorganisms isolated from infections are anaerobic bacteria, one of which is *Enterococcus faecalis* bacterium. It is considered to be the main cause of root canal abnormalities with a prevalence value of 77%.[5,6] *Enterococcus faecalis* is the dominant species of facultative anaerobic gram-positive cocci that exist in pairs, singles, short chains, oval or rounded egg-shaped.[7,8] *Enterococcus faecalis* can survive and multiply in the root canal with poor nutrient, high pH (alkaline) up to 11.5, and any help from other bacteria.[9] The resilience of *Enterococcus faecalis* to survive in an unfavorable root canal environment causes this bacterium to be a pathogen that leads to root canal treatment failure. The failure can be prevented by using an appropriate irrigation with low toxicity solution.[7] An ideal irrigation material should be non-toxic, able to dissolve organic and inorganic tissues, prevent smear layers formation during root canal preparation, and have antimicrobial properties.[4]

Chlorhexidine (CHX) 2% was suggested as a root canal irrigation agent because its antimicrobial effect can effectively protect the root canal after root canal treatment at that concentration. However, 2% chlorhexidine is not recommended as the first option for irrigation agents since it causes discoloration and allergic reactions when used repeatedly over a long period of time.[10,11] The damaging effects of these irrigation agents have encouraged people to search alternative treatments, such as herbal medicine. One herbal medicinal plant often utilized as traditional medicine by the community is saga leaves.[8]

Abrus precatorius Linn is known as cough medicine and treatment for stomatitis, pharyngitis, and tonsillitis.[12] According to research, it has been reported that saga leaves plant *(Abrus precatorius Linn*) have preclinical anti-inflammatory properties. Saga leaf extract contains flavonoids, terpenoids, tannins, alkaloids, and saponin compounds that are effective as antibacterials by inhibiting cyclooxygenase and lipooxygenase.[13,14] The antibacterial activity of saga leaves has been tested and proven effective against Streptococcus mutans bacteria, a gram-positive facultative anaerobic bacteria.[15] This study was conducted to determine the effect of different saga leaf concentrations extract in inhibiting *Enterococcus faecalis* bacteria growth.

METHODS

This was a laboratory experimental study with in-vitro post-test only control group design by giving different treatments to *Enterococcus faecalis* bacteria with saga leaf extract (*Abrus precatorius Linn*) in various concentrations with the well diffusion test method. The formation of inhibition zone was calculated in each treatment. This research was conducted at Aretha Medika Utama Laboratory, Bandung.

Plant determination

The saga leaves were obtained from a local herbal plantation in South Bogor sub-district, Bogor city, West Java, which was then identified at the Biology Laboratory, Padjadjaran University, Jatinangor, West Java, to obtain the determination of *Abrus precatorius Linn.*

Concentration Series Preparation

This study was treated with a positive control in the form of 2% chlorhexidine, negative control in the form of 100% DMSO and saga leaf extract (*Abrus precatorius Linn*) with concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%. Calculation of dilution of saga leaf extract solution and 100% DMSO were performed to make series of concentrations. The varying concentration of extracts used are as follows:

100% saga leaf extract: Stock solution

50% saga leaf extract: 500 µL stock solution + 500 µL DMSO 100% (Solution A)

25% saga leaf extract: 500 µL solution A + 500 µL DMSO 100% (Solution B)

12.5% saga leaf extract: 500 µL solution B + 500 µL DMSO 100% (Solution C)

6.25% saga leaf extract: 500 µL solution C + 500 µL DMSO 100% (Solution D)

3.125% saga leaf extract: 500 µL of solution D + 500 µL of 100% DMSO

Preparation of Saga Leaf Extract

This research was conducted at the Central Laboratory, Padjajaran University, Jatinangor, West Java. A total of one kilogram of saga leaves were dried and pulverized using blender to form fine powder. 287 grams of saga leaf powder were used in the first maceration process by soaking it with 1 litre of 96% ethanol solvent in a tightly closed

container. The soaking process was carried out for 72 hours and mixed occasionally. The saga leaf solution then proceed to the filtering stage using a filter paper to separate the solution. The filtrate was separated and placed in a glass bottle. The filtrate was then evaporated using vacuum rotary evaporator with a temperature of 45-50 °C; 50 rpm speed; 180-70 mbar pressure for roughly 5 hours until a thick saga leaf extract was obtained. Furthermore, the dilution process was carried out using 100% DMSO at a concentration of 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%. Fianlly, the ethanol extract of saga leaves with various concentrations were placed into closed sterile bottles and stored in the refrigerator at -20℃.

Phytochemical Analysis

Phytochemical analysis test was conducted to test the presence of bioactive compounds, such as tannins, flavonoids, alkaloids, steroids, triterpenoids, phenolics and saponins from the saga leaf extract. Alkaloids were identified using Dragendroff reagent. Flavonoids were identified using HCl+Mg, H_2SO_4, and NaOH 10%. Saponins were detected by heating method. Tannins and phenolics were identified using 1% and 5% $FeCl_3$ preaction, Steroids and triterpenoids were analyzed with H_2SO_4 + CH_3COOH. Qualitative results were expressed as (+) for presence and (-) for absence of phytochemicals.

Culture of *Enterococcus faecalis* bacteria

The sample in this study was *Enterococcus faecalis* bacteria (ATCC 29212) obtained from Aretha Medika Utama Laboratory Bandung. The study used 19 grams of Mueller Hinton Agar (MHA) and 10.5 grams of Mueller Hinton Broth (MHB) as the growth media which were measured using an analytical balance. Microwave was used to help dissolve the growth media in 500 mL of ddH2O. The next step was the sterilization process using an autoclave at 121^{o}C with a pressure of 1.5 atm for 20 minutes under anaerobic conditions. Colony suspension method was used to prepare the bacterial inoculum by inoculating *Enterococcus faecalis* colonies that had been cultured for 18-24 hours on MHA medium, into MHB medium. The solution's turbidity was adjusted according to the McFarland 0.5 standard solution's turbidity to produce an inoculum with a bacterial count of approximately 1-2×108 CFU/Ml. Pure culture of bacteria was taken as much as 1 ose and implanted on MHA by swab method.

Diffusion Test

The inoculation process on the test agar plates was carried out using the swab method by dipping a sterile cotton swab into the prepared bacterial suspension. The cotton swab was pressed against the tube wall to remove excess suspension, which was then applied to the surface of the MHA medium evenly. The inoculation was allowed to rest at room temperature for 3 to 5 minutes until the suspension was absorbed into the agar. After that, holes were made in the Mueller Hinton Agar (MHA) medium using 8 mm-diameter tips. Each holes were filled with 50 µL of different concentrations of saga leaf extract, starting with the 3.125%, 6.25%, 12.5%, 25%, 50%, 100%, the positive control 2% chlorhexidine and the negative control DMSO 100% respectively. In this study, the test agar wells were made for 3 repetitions. The agar plates were first incubated at 37^{o}C for 24 hours before measuring the diameter of the inhibition zone formed using a caliper.

Statistical Analysis

Data from the measurement of inhibition zone diameter were obtained and tested statistically using the Normality Test (Shapiro-Wilk), Homogeneity Test (Levene), Parametric Test (One Way ANOVA), Further Test (Post Hoc Test).

RESULTS

Phytochemical Examination

The extracted saga leaf plants were then subjected to qualitative phytochemical testing with the following test results:

Table 1. Phytochemical Test Results on Saga Leaf Extract (*Abrus precatorius Linn*)

Based on the results of phytochemical tests, only secondary metabolite compounds flavonoids appeared in low amounts (+) with 10% NaOH testing, while saponins, tannins, steroids, and phenolic appeared in moderate amounts (++).

Measurement of Inhibition Zone Diameter of Saga Leaf Extract against *Enterococcus faecalis*

In this study, antibacterial measurements were calculated from the inhibition zone diameter. The inhibition area measured is a clear zone where *Enterococcus faecalis* bacteria did not grow. The clear zone area was measured using a caliper in millimeters (mm). These are the following results:

Figure 1. The Zone of Inhibition of Saga Leaf Extract against *Enterococcus faecalis* shown by a clear area

Table 2: Results of Measurement of the Diameter of the Zone of Inhibition of Saga Leaf Extract against *Enterococcus faecalis* bacteria

Diagram 1. Comparison of Inhibition Zone Diameter of Saga Leaf Extract against *Enterococcus faecalis* bacteria

Based on the table and graphic above, it can be infered that saga leaf extract shows antimicrobial activity against *Enterococcus faecalis* bacteria characterized by the presence of inhibition zone diameter at 100%, 50%, 25%, 125%, 6.25% and 3.125% concentrations saga leaf extract. The largest inhibition zone was with 100% concentration with an average diameter of 9.46 mm, and the lowest inhibition zone was with 3.125% concentration with an average diamter of 1.26 mm. The level of inhibition shown by saga leaf extract is directly proportional to the level of concentration where higher extract concentration produces higher inhibition area.

DISCUSSION

Infering from the data, saga leaf extract shows an effect in inhibiting *Enterococcus faecalis* bacteria growth. The diameter of the inhibition zone grows from using the smallest to the largest concentration where the smallest inhibition zone happened at 3.125% concentration with 1.26 mm, whereas the largest inhibition zone was at 100% concentration at 9.46 mm diameter. The result can be explained due to higher concentration of saga leaf extract has more bioactive compounds contained. These bioactive compounds cause the inhibition zone to appear on the bacterial culture media.

The ability of saga leaf extract to inhibit the growth and kill *Enterococcus faecalis* bacteria is related to bioactive compounds that has antibacterials properties. The results of qualitative phytochemical tests on saga leaf extract used in this study showed the presence of phenolic compounds, tannins, saponins, steroids in moderate amounts; flavonoids in small amounts; and no alkaloid compounds were detected. There are factors that causes failure to detect alkaloid compounds such as soil type, soil pH, organic matter content, air temperature and rainfall in the area where the plant grows. These factors can cause content in the plants of each region to have different cadences.[16,17]

Phenolic compounds are said to prevent systemic inflammation by restoring redox balance by modulating the inflammatory response through mitigating the cytokine pathway to degrade oxidative stress. However, the actual mechanism of phenolic acid itself in inhibiting bacteria is not fully understood because it has a complex chemical structure. In some studies, it is hypothesized that phenolic acids can damage the electrochemical gradient of the mitochondrial membrane and prevent systemic inflammation.[18,19]

Saponin compounds are able to react with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall to form strong polymer bonds that causes porin damage.[20] Damage to porins will reduce the permeability of the bacterial cell membrane since it is the entrance and exit of the compound. It cause bacterial cells to lack nutrients, inhibited its growth and die.[20,21] This helps the entry of tannin and flavonoid compounds to enter bacterial cells.

Tannins activates reverse transcriptase, the adhesions of the microbial cell, DNA topoisomerase enzymes that will interfere with bacterial DNA synthesis, and also attack cell wall polypeptides, causing damage to bacterial cell wall.[22] All of this is possible because tannins have a target on the polypeptide wall of bacterial cells, resulting in incomplete cell wall formation and then bacterial cells will die.[20]

It is reported that steroids acts as antibacterial due to lipid membrane correlation and sensitivity to steroid compounds that show leakage in liposomes. Interaction between steroids and cell phospholipid membranes that are permeable to lipophilic compounds causes integrity of the membrane to decrease and changes to the morphology of the cell membrane, causing bacterial cells to become fragile and lysed.[23,24]

Flavonoids functions as antibacterial by inhibiting bacterial growth by forming complex compounds against extracellular proteins that disrupt the integrity of the bacterial cell membrane. The compound denatured the bacterial cell proteins and damaged the cell membrane to prevent reparation process.[25] Furthermore, bacterial mobility is also

inhibited by flavonoids due to the presence of flavonoid's hydroxyl groups that cause alterations in organic components and nutrient transport that lead to harmful effects on bacteria.[26]

In previous studies, saga leaf extract (*Abrus precatorius Linn*) was shown to have antibacterial activity by inhibiting several bacteria, both gram-negative and gram-positive bacteria, such as Streptococcus mutans bacteria which are included in the gram-positive facultative anaerobic bacteria group. Saga leaf extract (*Abrus precatorius Linn*) was proven to effectively work as an antibacterial at concentrations of 50%, 25%, 12.5%, 6.25%. It was also proven at 50% concentration saga leaf extract (*Abrus precatorius Linn*) that the inhibition zone was formed to inhibit Streptococcus mutans bacteria at a diameter of 10.3 mm. Therefore, the results of this research are comparable to the results of previous studies conducted, that shows ethanol extract of saga leaves (*Abrus precatorius Linn*) works effectively as an antibacterial.

CONCLUSION

Based on the results of the study, it can be concluded that saga leaf extract (*Abrus precatorius Linn*) is able to show antimicrobial activity against *Enterococcus faecalis* bacteria characterized by the presence of inhibition zones formed in different concentrations of saga leaf extract, starting at the smallest concentration of 3.125% to the largest concentration of 100%. The level of inhibition shown by saga leaf extract is directly proportional to the level of concentration, which means that the higher the concentration of the extract, the higher the inhibition produced. The level of inhibition of the extract is lower than that of chlorhexidine solution and is significantly different based on the statistical tests conducted.

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Bukti melakukan review yang pertama (23 Mei 2024)

Reviewer 1 report:
Comments to authors
Abrus Precatorius contains lethal toxin Abrin, a toxalbumin that inhibits protein synthesis causing cell death and tissue damage.
Hence it is necessary to determine from further investigation the toxicity of the active constituents and their side effects.

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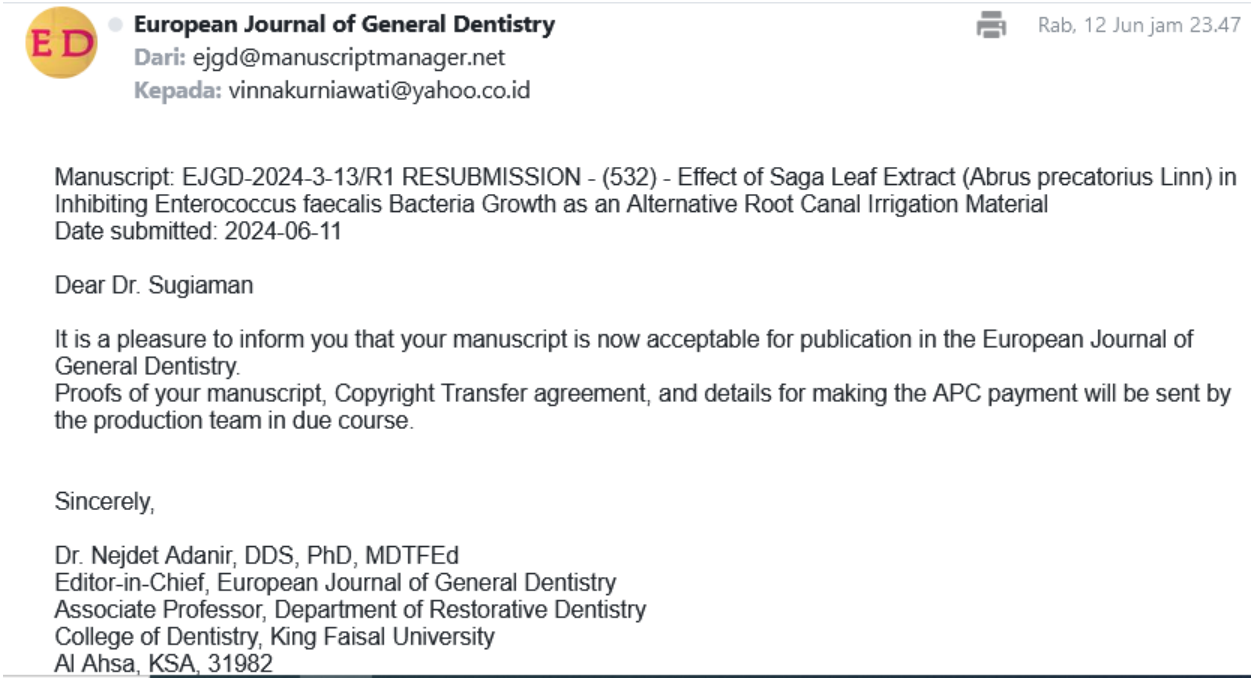

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