

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

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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

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Abstract

Objective This study aims to examine the antibacterial effect of saga leaf extract on *Enterococcus faecalis* bacteria, which causes root canal treatment failure.

Materials and Methods 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. Two percent chlorhexidine acts as the positive control, and 100% dimethyl sulfoxide as the negative control. The diameter of the inhibition zones was measured using the well diffusion test method.

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 analysis of variance), and further test (post hoc test).

Results 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.

Conclusion This study concludes that saga leaf extract has an antibacterial effect on the growth of *E. faecalis* bacteria.

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 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 reinfection 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 *E. 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

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material should be nontoxic, able to dissolve organic and inorganic tissues, prevent smear layers formation during root canal preparation, and have antimicrobial properties.⁴

Two percent chlorhexidine (CHX) 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% CHX 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 (*A. 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 lipoxygenase.^{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 *E. faecalis* bacteria growth.

Materials and Methods

This was a laboratory experimental study with in vitro posttest only control group design by giving different treatments to *E. faecalis* bacteria with saga leaf extract (*A. 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 subdistrict, Bogor city, West Java, which was then identified at the Biology Laboratory, Padjadjaran University, Jatinangor, West Java, to obtain the determination of *A. precatorius* Linn.

Concentration Series Preparation

This study was treated with a positive control in the form of 2% CHX, negative control in the form of 100% dimethyl sulfoxide (DMSO), and saga leaf extract (*A. 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, Padjadjaran University, Jatinangor, West Java. A total of 1 kg of saga leaves was dried and pulverized using blender to form fine powder; 287 g of saga leaf powder were used in the first maceration process by soaking it with 1 L of 96% ethanol solvent in a tightly closed container. The soaking process was performed for 72 hours and mixed occasionally. The saga leaf solution then proceeds 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 to 50°C; 50 rpm speed; 170–180 mbar pressure for roughly 5 hours until a thick saga leaf extract was obtained. Furthermore, the dilution process was performed using 100% DMSO at a concentration of 3.125, 6.25, 12.5, 25, 50, and 100%. Finally, the ethanol extract of saga leaves with various concentrations were placed into closed sterile bottles and stored in the refrigerator at –20°C.

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 Dragendorff reagent. Flavonoids were identified using HCl + Mg, H₂SO₄, and NaOH 10%. Saponins were detected by heating method. Tannins and phenolics were identified using 1 and 5% FeCl₃ reaction, and steroids and triterpenoids were analyzed with H₂SO₄ + CH₃COOH. Qualitative results were expressed as (+) for presence and (–) for absence of phytochemicals.

Culture of *Enterococcus faecalis* Bacteria

The sample in this study was *E. faecalis* bacteria (ATCC 29212) obtained from Aretha Medika Utama Laboratory, Bandung. The study used 19 g of Mueller–Hinton agar (MHA) and 10.5 g 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°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 *E. faecalis* colonies that had been cultured for 18 to 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 ~ 1 to 2×10^8 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 performed 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 MHA medium using 8-mm-diameter tips. Each hole was filled with 50 μ L of different concentrations of saga leaf extract, starting with 3.125, 6.25, 12.5, 25, 50, and 100%, the positive control 2% CHX, and the negative control DMSO 100%, respectively. In this study, the test agar wells were made for three repetitions. The agar plates were first incubated at 37°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 analysis of variance), and 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.

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 *E. faecalis* bacteria did not

grow. The clear zone area was measured using a caliper in millimeters (mm). The results are shown in –Tables 1 and 2, and –Figs. 1 and 2.

Based on the tables and figures above, it can be inferred that saga leaf extract shows antimicrobial activity against *E. faecalis* bacteria characterized by the presence of inhibition zone diameter at 100, 50, 25, 12.5, 6.25, and 3.125% concentrations of 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 diameter 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

Inferring from the data, saga leaf extract shows an effect in inhibiting *E. 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 *E. 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

Table 1 Phytochemical test results on saga leaf extract (*Abrus precatorius* Linn)

No.	Metabolite compounds	Test method	Test results
1	Alkaloid	Dragendorff reagent	–
2	Flavonoid	Concentrated HCl and Mg reagent	–
		2N H ₂ SO ₄ reagent	–
		10% NaOH reagent	+
3	Saponin	Heated	++
4	Tannin	1% FeCl ₃ reagent	++
5	Steroid	Concentrated H ₂ SO ₄ reagent and anhydrous CH ₃ COOH	++
6	Triterpenoid	Concentrated H ₂ SO ₄ reagent and anhydrous CH ₃ COOH	–
7	Phenolic	5% FeCl ₃ reagent	++

Table 2 Results of measurement of the diameter of the zone of inhibition of saga leaf extract against *Enterococcus faecalis* bacteria

Treatment	Inhibition zone diameter (mm)			Mean	Standard Deviation	RSD
	P 1	P 2	P 3			
Positive control (2% chlorhexidine)	17.50	17.06	15.10	16.55	1.28	7.72
Negative control (dimethyl sulfoxide 100%)	0.00	0.00	0.00	0.00	0.00	0.00
100% saga leaf extract	9.12	9.69	9.57	9.46	0.30	3.18
50% saga leaf extract	8.08	7.59	7.50	7.72	0.31	4.04
25% saga leaf extract	5.50	4.56	5.06	5.04	0.47	9.33
12.5% saga leaf extract	3.45	3.01	4.09	3.52	0.54	15.44
6.25% saga leaf extract	2.12	2.15	2.19	2.15	0.04	1.63
3.125% saga leaf extract	1.43	1.33	1.08	1.26	0.25	19.72

Abbreviation: RSD, relative standard deviation.

11) hway 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 causes 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 act 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 function 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

Abrus precatorius contains the lethal toxin Abrin, a toxalbumin that inhibits protein synthesis leading to cell death and tissue damage.³ It is necessary to investigate further the toxicity of its active constituents and side effects.

Abrin is a type-II ribosome-inactivating protein from *A. precatorius* seeds that is similar in structure and properties to ricin. It is classified as a Category B bioterrorism warfare agent. Abrin is 75 times more toxic than ricin, and is classified as a Category B biological warfare agent in the United States due to its widespread source, ease of preparation, and lethality. Abrin has a molecular weight of ~63 kDa, consisting of A chain with N-glycosidase activity and a galactose-

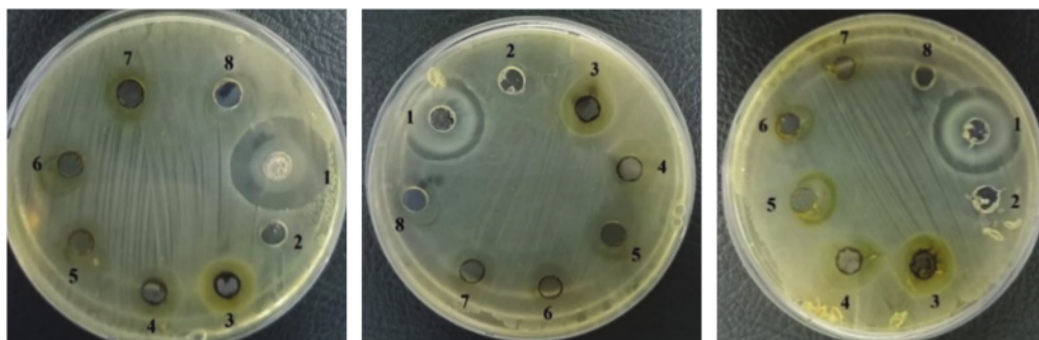


Fig. 1 The zone of inhibition of saga leaf extract against *Enterococcus faecalis* shown by a clear area.

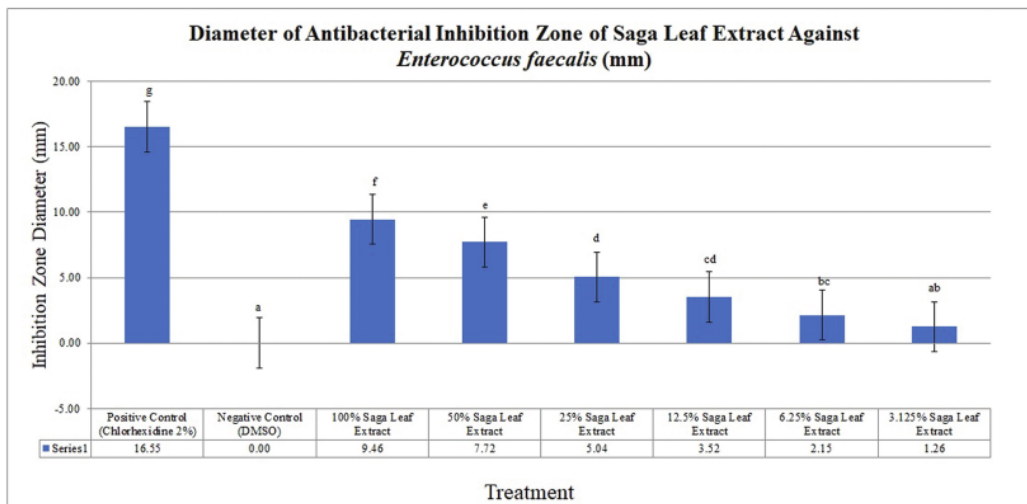


Fig. 2 Comparison of inhibition zone diameter of saga leaf extract against *Enterococcus faecalis* bacteria.

specific lectin B chain. The structure of the abrin B chain consists of lectin that binds to the β -D-galactoside portion of the cell surface and mediates the internalization and endocytosis of the entire toxin into the host cell. There are four isoforms of abrin: abrin-a, abrin-b, abrin-c, and abrin-d. Abrin-a and abrin-d are the most toxic. Abrin-b and abrin-c show weak B-chain lectin activity.^{1,2}

After entering the cell, the A chain can hydrolyze the N-glycosidic bond of the 28S ribosomal RNA of eukaryotic cells and catalyze depurination, causing ribosome inactivation and inhibits protein synthesis, therefore causing cell apoptosis. Due to its high toxicity, consumption by human will cause death due to multiple organ failure. Abrin is highly toxic, with an estimated fatal dose of 0.1 to 1 $\mu\text{g}/\text{kg}$. It has caused death after intentional or accidental poisoning. Clinically, abrin is considered an immunotoxin that can target cancer cells and can be delivered to the tumor site after combining with antitumor drugs.¹⁻⁴

At the cellular level, abrin inhibits protein synthesis, leading to cell death. Many of the features observed in abrin poisoning can be shown by abrin-induced endothelial cell damage, which leads to increased capillary permeability. This results in fluid and protein leakage and tissue edema (vascular leak syndrome). The most reported cases of human poisoning involve the consumption of jequirity beans, most of which cause gastrointestinal toxicity.²

In previous studies, saga leaf extract (*A. precatorius* Linn) was shown to have antibacterial activity by inhibiting several bacteria, both gram-negative and gram-positive bacteria, such as *S. mutans* bacteria which are included in the gram-positive facultative anaerobic bacteria group. Saga leaf extract (*A. precatorius* Linn) was proven to effectively work as an antibacterial at concentrations of 50, 25, 12.5, and 6.25%. It was also proven at 50% concentration of saga leaf extract (*A. 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 (*A. precatorius* Linn) works effectively as an antibacterial.

Conclusion

Based on the results of the study, it can be concluded that saga leaf extract (*A. precatorius* Linn) is able to show antimicrobial activity against *E. 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 CHX solution and is significantly different based on the statistical tests conducted.

Conflict of Interest

None declared.

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