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The Minimum Inhibitory Concentration (MIC) And Minimum Bactericidal Concentration (MBC) Of Sambiloto Leaf Extract Against Enterococcus Faecalis

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ABSTRACT

Background: Enterococcus faecalis is a bacterium that causes failure of the root canal treatment. Sodium hypochlorite is often used as irrigation, but it caused irritate the tissue. The chemical compounds in sambiloto leaf are tanin, saponin, alkaloid, flavonoid and andrographolide which have antibacterial potency.
Purpose: To determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of sambiloto leaf extract against Enterococcus faecalis.
Methods: Sambiloto leaf extract was made up to 5 concentrations which are 1,56%, 2,09%, 2,61% and 3,125%. 5 tubes consist of BHIB and 0,1 ml suspension of bacteria, 1 tube consist only with BHIB. 5 tubes added with sambiloto leaf extract in variated concentrations. The tube was incubated in anaerobic jar at 37o C for 24 hours. Then the suspension of bacteria was subcultured in nutrient agar. Minimum inhibitory consentration (MIC) and minimum bactericidal concentration (MBC) counted manually.
Results: Sambiloto leaf extract at a concentration of 1,56% showed inhibition of bacterial growth while the concentrations of 2,09% showed no bacterial growth.
Conclusion: Sambiloto leaf extract has antibacterial effect at minimum inhibitory concentration of 1,56% and minimum bactericidal concentration of 2,09%

Keywords: Enterococcus faecalis, minimum bactericidal concentration (MBC), minimum inhibitory

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INTRODUCTION

The most common bacteria that causes failure of the root canal treatment is *Enterococcus faecalis.* This study shows the existence prevalence of *Enterococcus faecalis* in the root canal by 24-77%. Bacteria *Enterococcus faecalis* has the ability to conduct an attachment and invasion into the dentine tubules, change the host immune system, be resistant to medicaments, form biofilms, the ability to compete with the other bacteria, virulent factors: *aggregation substance, surface adhesion, lytic enzymes, cytolisin, lipoteichoic acid*. Those factors are able to make bacteria *Enterococcus faecalis* survive in an infected root canal and cause failure of the root canal treatment [1,2]. These bacteria are categorized as gram-positive, anaerobic facultative, do not form spores, consist of short and paired chains, asymptomatic bacteria that cause infections, can grow at a temperature of 60o C [3]. This bacteria has the ability to survive in certain conditions in which the other other is lethal in such conditions. Those conditions include the ability to survive in high pH, the influence from the root canal medicament and high temperature[4].

The success of root canal treatment depends on several things, including the accuracy of diagnosis and treatment plans, good disinfection, selection of the used instruments and materials, treatment procedures (preparation, use of antimicrobials, root canal filling) and rehabilitation. The root canal treatment can be said to be successful if there is no pain caused by inflammation of the root canal and periapical, good healing process and optimal return of function [5,6].

The inappropriate selection of irrigation materials can make the bacteria to be left in the root canal, invade tissue, and cause re-infection. Irigation is one of the princples of endodontic treatment, namely *triad endodontic treatment*.

The function of irrigation is rinsing and dissolving infected hard and soft tissue deposits, elimination of microorganisms, necrotic tissue and cleaning dentine fragments from infected root canal [7,8].

In the selection of irrigation materials, it is needed the operator knowledge about the characteristic of various irrigation material solutions. An ideal irrigation must fulfill the requirements of a broad antimicrobial spectrum, be able to penetrate deep dentinal tubules, dissolve necrotic tissue, dissolve inorganic material, biocompability, non-irritating, easy to use and, affordable price [9].

Sodium hypochlorite (NaOCl), *Ethylene-diamine-tetra-acetic-Acid* (EDTA), *Ultrasonic activation of sodium hypochlorite* and *Chlorhexidine* (CHX) are the types of irrigation materials used for root canal treatment. The most frequently used irrigation material is *sodium hypochlorite* [10,11].

Various studies have been conducted in order to find the alternative root canal irrigation materials using a natural or combination of materials. Sambiloto leaf has been widely used in Asia for herbal medicine. This leaf is one of the traditional medicinal plants which has been tested for its efficacy. In Indonesia, sambiloto leaf can be found on the market in a single dosage form, combined with other natural ingredients in the form of tablets or herbal preparations [12,13]. The society knows sambiloto leaf as bitter plant which has various efficacies. The compounds in sambiloto leaf include tannin, saponin, alkaloids, flavonoids, and andrographolide which have various potential including antibacterial, antifungal, antiviral, anti-inflammatory and antipyretic properties [14].

The aim of this study is to discover the minimum

inhibitory concentration and minimum bactericidal concentration of sambiloto leaf extract on the growth of *Enterococcus Faecalis.* Previous studies on the determination of minimum inhibitory concentration and minimum bactericidal concentration have been conducted, as follows 100%, 50%, 25%, 12,5%, 6,25%, 3,125%, 1,56% and 0,78%, are obatined the minimum inhibitory concentration of 1,56% and minimum bactericidal concentration (MBC) of 3,125%. This becomes the basis of further research to determine the accuracy of the minimum inhibitory concentration and minimum bactericidal concentration with a concentration of 1.56%, 2.09%, 2.61% and 3.125%.

MATERIALS AND METHOD

This research is an experimental laboratory study with a *post test only group design* research design. The number of samples were 6 groups with 7 repetitions. The sambiloto leaf extraction process is conducted at Balai Materia Medica, Batu, Malang, East Java. The examination to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was conducted at the Research Center of the Faculty of Dentistry, Universitas Airlangga. The tools used for the extraction process are knives, 1000 ml measuring cups,

glass funnels, bottles, analytical scales, closed jars, blenders, water baths, erlenmeyer tubes, digital shakers, rotary vacuum evaporators, beaker glass, and alcohol meters. The tools for the examination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are test tube rack, test tube, petridish, spreader, ose, micropipette, methylated brander, anaerobic jar, incubator, quebec and colony counter. The materials used are sambiloto leaf, 70% etabol, bacteria *Enterococcus faecalis* in the media of *Brain Heart Infusion Broth* (BIHB), media *nutrient agar*, and *gas generating kit*.

The extraction process of sambiloto leaf is conducted by weighing that leaf for 300 g, and then is dried. The powder production is conducted by destroying dried sambiloto leaf and then moistened with 70% ethanol solvent until submerged. Stirring was done using a digital shaker with a speed of 50 rpm for 24 hours. The sambiloto extract is filtered with a filter cloth. The result of sambiloto leaf extract is put into a measuring cup, evaporated with a rotary evaporator, and then evaporated again above the water bath until the alcohol content is completely gone. The liquid extract of the sambiloto leaf was obtained with a concentration of 100% as much as 55 ml. The production of sambiloto leaf extract formulation with concentration of 3,125%, 2,61%, 2,09%, 1,56% uses the comparison of the leaf extract with BHIB: mixture of 3.125 ml sambiloto leaf extract + 6.875 ml BHIB, mix 2.61 ml sambiloto leaf extract + 7.39 ml BHIB, mix 2.09 ml sambiloto leaf extract + 7.91 ml BHIB, mix 1.56 ml of sambiloto leaf extract + 8.44 ml BHIB.

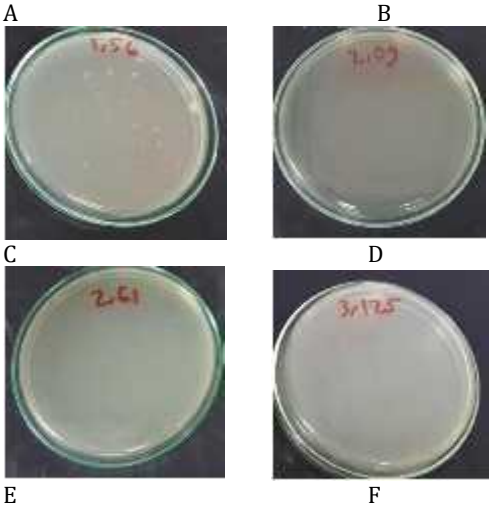
The sample group was divided into 5 groups namely the *Enterococcus faecalis* bacteria group by giving the sambiloto leaf extract concentrations of 1.56%, 2.09%, 2.61%, 3.125%, positive control and negative control. All the tube reactions are filled with the media of *Brain Heart Infusion* (BHIB) as much as 5 ml along with the sambiloto leaf extract, except the positive control contains BHIB media and bacteria *Enterococcus faecalis* as well as negative control filled BHIB media, and then anaerobically incubated in an incubator for 24 hours at 37 ° C. Afterward, the observatio of the number of colonies that grew with a subculture of bacteria as much as 0.1 ml from each tube and positive control on *agar nutrient* media is conducted. The *agar nutrient* media were incubated for 24 hours at 37 ° C in an incubator. Determination of MIC and MBC is done by counting the number of *Enterococcus faecalis* bacterial colonies growing on the *nutrient agar* media manually and expressed in units of CFU / ml.

RESULT

Bacterial colonies were calculated at a concentration of 1.56% which indicates the growth of the colony, this concentration is the minimum inhibitory concentration (MIC). At a concentration of 2.09% there was no bacterial growth, it can be concluded that at a concentration of 2.09% was the minimum bactericidal concentration (MBC). The results of the calculation of the colony are shown in the table 1.

The results of the colonies calculation in further studies positive control of 160 colonies. In the negative control there was no colony growth. A concentration of 1.56% obtained 19 colonies. Concentrations of 2.09%, 2.61% and 3.125% did not obtain colony growth (Picture 1).

From these results it can be determined that the minimum inhibitory concentration (MIC) of sambiloto leaf extract on the growth of bacteria *Enterococcus faecalis* at a concentration of 1.56% and the minimum bactericial concentration (MBC) of sambiloto leaf extract on the growth of bacteria *Enterococcus faecalis* at a concentration of 2.09%.



Picture 1.. Subculture of bacteria *Enterococcus faeca- lis*

in the *nutrient agar*.
(A) positive control;
(B) negative control;
(C) concentration of 1,56%;
(D) concentration of 2,09%;
(E) concentration of 2,61%;
(F) concentration of3,125%.

Table 1. The result of colony calculation of *Enterococcus faecalis*

Replication	Concentration					
	3,125%	2,61 %	2,09 %	1,56 %	K(+)	K(-)
1	0	0	0	19	166	0
2	0	0	0	18	157	0
3	0	0	0	20	172	0
4	0	0	0	21	165	0
5	0	0	0	16	154	0
6	0	0	0	17	158	0
7	0	0	0	20	149	0

The data analysis was performed in the 1.56% concentration group and positive control using *the Kolmogorov-Smirnov test*, *the Levene test* and *the Independent T-Test.* Based on the results of the normality test, the *Kolmogorov-Smirnov* statistical test results showed a positive control group and a concentration of 1.56% having a significance value greater than 0.05 (p> 0.05). It can be concluded that the data obtained are normally distributed.

Afterward, the *Independent T-Test* obtained 0.000 (p <0.05), so it can be interpreted that there is a significant difference between positive control and 1.56% concentration. This is consistent with the results of research that has been done. It can be concluded that the administration of sambiloto leaf extract affects the growth of bacteria *Enterococcus faecalis* with a minimum inhibitory concentration (MIC) of 1.56% and a minimum bactericidal concentration (MBC) of 2.09%.

DISCUSSION

From the results of statistical test, there are significant differences between positive control and 1.56% concentration. Sambiloto leaf extract has chemical contents such as saponins, alkaloids, flavonoids and tannins. The other chemical ingredients are paniculin and kalmegin. The main active component of sambiloto leaf is andrographolide which is proven in several studies to have antimicrobial effects on various microbial organisms [15,16].

The tannin content in the sambiloto leaf extract which has antibacterial properties activates the attachment of microbial cell walls, microbial enzyme production, and completely interferes with cell wall protein transport.

The cell wall that is not formed properly causes osmotic pressure and physical pressure, so that bacteria lysis and die [17].

The formation of complex compounds between flavonoids and extracellular proteins causes the dissolution of compounds in the bacterial cell wall so that the bacterial cell membrane becomes damaged. The damaged bacterial cell membrane can not lead to the return of the cell membrane as before. In addition, the mechanism of flavonoids can also inhibit the process of energy metabolism [18,19]. As a result, bacteria cannot survive because they do not produce energy from metabolic processes.

Saponins reduce the surface tension of the bacterial cell wall, then diffused into the outer membrane and eventually the cell wall becomes vulnerable. Vulnerable cell walls bind to the cytoplasmic membrane, then the stability of the cell membrane is reduced. As a result, cytoplasm leaks and bacterial cells die [20].

The antibacterial mechanism of alkaloid compounds is by damaging the components of peptidoglycan in the bacterial cells so that the bacterial cell wall layer is not formed intact and causes cell death [21]. The damage of the cell wall will cause inhibition of bacterial cell growth and eventually the bacteria will die.

Andrographolide compounds work by damaging bacterial cell membranes. Cell membrane which has been damaged will occur a leakage marked by the release of macro molecules such as proteins and nucleic acids so that the biosynthesis of specific enzymes in bacteria is inhibited. The function of specific enzymes in bacteria functions in the process of metabolic reactions. The presence of specific enzyme biosynthetic disorders in bacteria can affect bacterial growth [22].

The results of the calculation of Enterococcus faecalis colonies number showed that the higher the concentration of the bitter sambiloto extract, the smaller the growth of the colony. With the presence of chemical compounds in the sambiloto leaf extract such as tannins, flavonoids, saponins, alkaloids and andrographolide have a mechanism of action as an antibacterial against the bacteria Enterococcus faecalis.

The conclusion of this research is the sambiloto leaf extract can inhibit and kill the bacteria Enterococcus faecalis.

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