INHIBITORY EFFECT OF INDIAN LILAC LEAF EXTRACT (*Azadirachta indica*) ON THE GROWTH OF *E. FAECALIS* ATCC 29212

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ABSTRACT

This study aims to determine the inhibition of water-based Indian lilac leaf extract (*Azadirachta indica*) against *Enterococcus faecalis.* This study is a laboratory experimental conducted using the disc. There were 24 samples used in the form of cultures of *E. faecalis* in Muller Hilton Agar (MHA) media. Variations in the concentration of Indian lilac leaf extract treatment are 80%, 100%, NaOCl 2.5% (positive control), and sterile aquades (negative control). The mean inhibition of neem leaf extract concentrations of 80% and 100% was 0 mm, while the positive control NaOCl 2.5% was 0.85 mm.Water-based Indian lilac leaf extract contains antibacterial properties but no inhibition against Enterococcus faecalis bacteria.

Keywords: Root canal treatment, *E. faecalis*, biofilm, *Azadirachta indica*

1. INTRODUCTION

The existence of the pulp is very important, because the pulp tissue that will determine whether a tooth is still vital. in the event of mechanical trauma, the contamination of microorganisms through dental caries, cracks, and dentin tubules will cause an inflammation which leads to discomfort to the patients. If a pulp has necrotic, the patient does not respond to vitality tests, which is an indication that root canal treatment is necessary.

Root canal treatment (RCT) is one of the endodontic treatments that serves to keep the teeth in the oral cavity as long as possible. The results of RCT depend on proper instrumentation, irrigation, and root canal filling. Irrigation plays an important role in endodontic treatment, as it is the only way to clear areas of root canal walls that mechanical instrumentation cannot reach.Click or tap here to enter text.Failure in RCT could be caused by a variety of factors. One of them is bacterial persistence. Bacterial persistence is caused by microorganisms that are resistant to intracanal antibacterial, so that the bacteria can survive in root canals that have been treated endodontics. The highest incidence of RCT failure is due to repeated infection of facultative anaerobic bacteria. Several culture and molecular biology studies have revealed that *E. faecalis* is the most frequent species found in root canals of teeth that have been treated endodontically, with prevalence values reaching 90%.[1] In fact, *E. faecalis* bacteria are often found in teeth that had undergo RCT with several visits, therefore, it can be said that *E. faecalis* bacteria is the cause of secondary infection.

Sodium hypochlorite is the most used irrigation agent due to its antibacterial function and ability to dissolve biofilms, vital pulp tissue, organic components of dentin, and tissue necrosis. Sodium hypochlorite is also the most effective broad-spectrum irrigation agent against biofilms including *E. faecalis*. Sodium hypochlorite has several adverse events such as its unpleasant taste and toxicity. The risk of sodium hypochlorite toxicity may increase with higher concentrations used. Conversely, at low concentrations, the ability to dissolve tissues and their antibacterial effectiveness will certainly also decrease.

To overcome this problem, it requires other alternatives, such as the use of extracts from plants, one of which is Indian lilac (*Azadirachta indica*). Indian lilac is a plant that grows widely in tropical and subtropical regions. Indian lilac contains natural antibacterial ingredients such as triterpenoids, flavonoids, saponins, tannins, phenols, and steroid. There are studies that state that the antibacterial effect of Indian lilac leaf extract has been proven effective in inhibiting the growth of *E. faecalis.* But the antibacterial effect depends on the extraction base. Water-based Indian lilac leaf extract has antibacterial effects but requires higher concentration compared to alcohol-based extracts. The purpose of this study was to find the effective concentration of water-based Indian lilac leaf extract antibacterial against the growth of *Enterococcus faecalis.*

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2. METHODS

Type of research is experimental laboratory in vitro with post-test with control design. The research was carried out at the Herbal Laboratory of Yarsi University and the Research Centre for Science and Technology during May-June of 2023 with details of the Herbal Laboratory of Yarsi University for the manufacture of extracts and the Research Centre for Science and Technology for testing antibacterial effects.

Experimental Research Laboratory grown in microplate. The research population was *E. faecalis* bacteria and the samples were *E. faecalis* ATCC 29212. The samples were divided into 4 groups, namely the Indian lilac leaf ethanol extract treatment group (concentrations of 80% and 100%), positive control (NaOCl 2.5%), and negative control (distilled water). Each group had 6 repetitions in one plate. The research was repeated on other plates on different days to confirm the consistency of the data. This number of repetitions was adjusted to the number of reps that was acceptable and prevalent in cell-based experiments including antibacterial experiments, i.e. at least 4 repetitions per group, a greater number allowed to see the consistency of treatment results and if resources are adequate.

The tools used in this research were oases, racks and test tubes, 250 mL beakers, 25 mL measuring cups, Erlenmeyer flasks and 100 ML measuring flasks, scales, aluminum foil, autoclaves, incubators, oven, electric stove, stirring rods, Bunsen burner, 6mm paper discs, calipers, 1 mL, 5 mL measuring pipettes, 10 mL suction rubber, 1 mL volume pipette, funnel, micropipette, yellow tip and petri dish. The materials used were cultures of *E. faecalis* ATCC 29212 bacteria, dried Indian lilac leaves, distilled water, and 2.5% of NaOCl.

The research began with the preparation of maceration method extract with water solvent with a ratio between Indian lilac leaf powder and solvent is 1: 8 and soaked for 48 hours, in a closed space with shaker. After 48 hours the sample was filtered, and the filtrate obtained was then evaporated using a rotary evaporator at 40^{o}C until a thick extract was obtained.[20,25] Thick Indian lilac leaf extract was diluted by adding distilled water.

The research samples were prepared by growing *E. faecalis* ATCC 29212 bacteria in Muller Hilton Agar (MHA) medium and incubated for 48 hours in an anaerobic environment at 37^{0}C. The formation of colonies on MHA was characterized by when the presence of MHA became cloudy. The subculture results were made into a bacterial suspension according to McFarland standard 0.5 (~1.5 x 108 CFU/mL) with a physiological solution of 0.9% NaCl. The inhibition test of bacterial formation is carried out by dripping some treatment liquid onto disc papers. The disc papers were dripped with 80%, 100%, positive control NaOCl 2.5%, and negative control using micropipettes. The disc papers that had been dripped with extracts and control materials were then placed on the surface of Muller Hilton Agar (MHA) media that has been smeared with bacterial suspension. Incubated 37^{o}C for 24 hours anaerobically. The clear zone/inhibitory zone is measured using a caliper.

The data analysis using descriptive analysis. This analysis aims to determine the characteristics of the research data. The data to be calculated is the average, standard deviation, and median of the data.

3. RESULTS AND DISCUSSION

The analysis carried out was to conduct descriptive statistical analysis to determine the characteristics of the data from the research that has been done. The descriptive statistics calculated are the mean, standard deviation, and median data. Results of descriptive statistical analysis (Table 1):

Table 1. Mean, Median, and Standard Deviation of diameter of inhibition zone *E. faecalis* data

Treatment	***E. faecalis* (mm)**						Mean	Median	SD
	1	2	3	4	5	6			
Indian lilac leaf extract (80%)	0	0	0	0	0	0	0	0	0
Indian lilac leaf Extract (100%)	0	0	0	0	0	0	0	0	0
C + (NaOCl 2,5%)	1,06	1,11	0,64	0,7	0,98	0,63	0,85	0,84	0,22
C – (sterile aquadesl)	0	0	0	0	0	0	0	0	0

Based on the data from the diameter of inhibition zone test results, the highest is in the C + group with an average of 0.85 mm, a median of 0.84 mm, and a standard deviation of 0.22 mm. Based on the treatment of Indian lilac leaf extract, there is no increase in the diameter of the inhibitory effect so that the mean, median, and standard deviation is 0 mm.

This study conducted an inhibitory power test of Indian lilac leaf extract (Azadirachta indica) extracted with water solvent to determine its effectiveness in inhibiting Enterococcus faecalis ATCC 29212 bacteria. The Indian lilac leaves used come from Deunong Village, Darul Imarah District, Aceh Besar Regency, Aceh Province. Antibacterial contained in Indian lilac leaves can only be used after going through the extraction process. The extraction method used in this study is maceration using water solvents (distilled water). Maceration was chosen because it is practical and safe for compounds that are thermolabile (not heat resistant)

The extraction process starts from Indian lilac leaves that have been dried by aerating (without sunlight) at room temperature mashed using a blender to become powder. Indian lilac leaf powder is soaked with distilled water in a closed container in a ratio of 1:8 accompanied by shaker for 2 days. The results of the bath are filtered using filter paper so that filtrate is obtained. The filtrate is evaporated using a rotary evaporator at 40^{o}C until a viscous extract is obtained. To obtain a concentration of 80% diluted using distilled water.

Testing of antibacterial activity is carried out by disc method. The disc paper is cut into circles with a diameter of 6 mm and then dripped with Indian lilac leaf extract with a concentration of 80%, 100%, C+, and C-. paper that has been dripped extracts and control materials are placed on MHA media. Make sure the paper has been spaced and the paper has been in good contact with the agar media. Furthermore, the medium was observed for 24 hours on an anaerobic atmosphere. The diameter of the clear zone indicates that the test material contains antibacterial properties. The diameter of the clear zone is measured using a caliper in mm units.

The results of antibacterial activity tests of water-based Indian lilac leaf extract in the study showed that at concentrations of Indian lilac leaf extract 80%, 100%, and C- did not show any inhibitory power for the growth of *E. faecalis* seen from the large clear zone formed around the paper disc.

According to David and Stout in 1971, antibacterial power can be divided into four criteria by looking at the clear zone formed around the paper disc. The assessment of the inhibitory zone is seen from the results of diameter measurements. If the diameter of the inhibitory zone ≤ 5 mm is categorized as weak, the diameter of the inhibitory zone of 5-10 mm is categorized as medium, the diameter of the inhibitory zone of 10-20 mm is categorized as strong, and the diameter of the inhibitory zone ≥ 20 mm is categorized as very strong. Referring to this criterion of Indian lilac leaf extract (*Azadirachta indica*) concentration 80%, 100%, and NaOCl concentrations of 2.5% have weak antibacterial effect.

The antibacterial effectiveness of NaOCl 2.5% is influenced by concentration, temperature, and application. Greater concentrations of NaOCl have greater tissue dissolving ability.[1] The effective level of irrigation material can be increased by activating the irrigation material either manually or with the help of tools.

The effectiveness of the inhibitory effect of an extract can be influenced by the type of solvent used, the shape of the sample before extraction (fresh / dried), the length of the soaking process, storage time, harvest age, and sterility of the material used as an extract.This extract was made on March 27, 2023, phytochemical test on April 3, and diameter of inhibition zone test on May 9, 2023. According to research conducted by Seja in 2018 showed that the effectiveness of the inhibitory power of an extract decreased on day 3. The longer the storage, the more microorganism activity will occur, which ultimately leads to spoilage.

This research is in line with research conducted by Susmitha in 2013 that water-based Indian lilac leaf extract contains antibacterial ingredients, but the effectiveness of water-based Indian lilac leaf extract is not as effective as alcohol-based Indian lilac leaf extract.18 This study is not in line with Kumar's assertion that water-based Indian lilac leaf extract has the same effectiveness as alcohol-based Indian lilac leaf extract.

Based on the discussion above, the hypothesis in this study, namely "There is inhibition of water-based neem leaf extract against *Enterococcus faecalis*" is rejected. The shortcomings in this study are the length of time between making the extract and carrying out the DDH test so that it can affect the effectiveness of this Indian lilac leaf extract.

4. CONCLUSION

Based on the results of research and data analysis regarding the antibacterial effectiveness of Indian lilac leaf extract against *Enterococcus faecalis* as an alternative to root canal irrigation that has been carried out, it is found that water-based neem leaf extract contains antibacterials (flavonoids, phenols, and tannins) but there is no inhibition effect on *Enterococcus faecalis*.

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