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Bioactivity of Lerak Fruit Extract (*Sapindusrarak DC*) as an Endodontic Irrigants to Inhibition the *Fusobacteriumnucleatum* virulence and Relate to the Fracture Resistance of Root Canal

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Abstract:-The irrigation solutions most often used in the endodontic treatment are NaOCl 2.5% and EDTA 17%. However, these two irrigation solutions still have shortcomings. One of the natural ingredients currently being developed as an irrigation solutions alternative is Lerak fruit extract. The results showed that the Lerak fruit (*Sapindusrarak DC*) extract solution was antibacterial, antifungal, cleaned root canal smear layer, and more biocompatible than the NaOCl solution. *F.nucleatum*was the most common bacteria found in teeth with root canal infections. This study aimed to determine the effect of Lerak fruit extract in inhibiting the *Fusobacteriumnucleatum* development through hydrophobicity, destructive enzyme expression, and fracture resistance. This experimental post-test only group design, started Lerak extract with ethanol as a solvent. 25%, 12.5% and 6.25% Lerak fruit extract, 2.5% NaOCl + EDTA 17%, hydrophobicity activity and destructive enzyme expression. Fracture resistance test with Universal Testing Machine (UTM). The results showed that Lerak fruit extracts of 6.25%, 12.5% , and 25% had better inhibition against the hydrophobicity of *F.nucleatum*than 2.5% NaOCl + EDTA 17% solution. The Lerak fruit extract ability to suppress the expression of the destructive enzyme *F.nucleatum*was equal to 2.5% + EDTA 17%. The fracture resistance of teeth irrigated with 12.5% and 25% Lerak fruit extracts was better than 2.5% NaOCl + EDTA 17% solution. In conclusion, Lerak fruit extract inhibited the hydrophobicity of *F. nucleatum*, suppressed the expression of the destructive enzyme *F. nucleatum*, and had a better effect on tooth fracture resistance than NaOCl 2.5% + EDTA 17%.

Keywords:- *Fracture resistance, Fusobacteriumnucleatum, Root canal, Sapindusrarak DC, Virulence.*

I. INTRODUCTION

The interaction and production of toxins by bacteria in the root canal can lead to endodontic infection. *Fusobacteriumnucleatum* (*F.nucleatum*) includes obligate anaerobic gram-negative bacteria and rods, often found in root canal infections with apical periodontitis, acute apical abscesses, post-endodontic infection, and pulp necrosis (Jhajharia, 2019). The factor affecting *F. nucleatum* with host cells is hydrophobicity at the beginning of attachment. The hydrophobicity interaction is significant because it is one of the main drivers of several phenomena in forming protein-protein or protein-ligand bonds (Canales, 2017).Bacteria produce and release several enzymes that are essential in bacterial pathogenicity. Enzymes produced by *F.nucleatum*bacteria such as hyaluronidase, elastase, fibrinolysin, hemolysin, chondroitin sulphatase, phosphatase, B-lactamase can potentially damage parent tissue and can act as a spreading factor (Flores-Díaz et al., 2016).

Root canal treatment eliminates all vital or necrotic pulp tissue and microorganisms and prevents recurrent infections. One of the root canal treatment goals can be achieved by chemomechanical cleaning and shaping. Chemomechanical preparation involves mechanical instrumentation using endodontic instruments and chemical cleaning of root canals with a root canal irrigation material (Saoud et al., 2015). However, the root canal irrigation material is one of the factors affecting fracture tendency. It has been reported that several chemicals used for endodontic irrigation can cause changes in dentin's chemical composition, affecting the microhardness, permeability, and solubility of dentin (Verma et al., 2019). Due to the weaknesses of these irrigation materials, it is necessary to develop natural materials as alternatives to root canal irrigation that meet the requirements for root canal irrigation (Neelakantan et al., 2018).

One of the natural ingredients that can be developed as a root canal irrigation material is Lerak fruit (*Sapindusrarak DC*). Pharmacological properties of Lerak include antifungal, bactericidal, and anti-inflammatory properties. The active components of Lerak fruit consist of saponins, alkaloids,

polyphenols, and flavonoids.[9]In the development of Lerak extract as a root canal irrigation material. It is known that 0.01% Lerak extract has an antibacterial effect against Streptococcus mutans. The antifungal product against Candida albicans is better than NaOClat 5%, *Fusobacteriumnucleatum* at a concentration of 0.25%, *Porphyromonasgingivalis*, and *Enterococcus faecalis* at a concentration of 25% (Nevi Yanti and Prasetia, 2017).

The objective of this study was to know the bioactivity of Lerak fruit extract (*Sapindusrarak DC*) against the pathogenesis of *Fusobacteriumnucleatum* bacteria, which will be studied with hydrophobicity activity tests and destructive enzyme expression tests. In addition, a dentin fracture resistance test was carried out to see the clinical effect on the root canal.

II. MATERIAL AND METHODS

A. Extraction of Lerak fruit

The Lerak fruit was extracted by using 70% ethanol. The Lerak fruit is obtained from mandailing Sumatera Utara, Indonesia, washed and weighed, and the pulp is cut with a width of ± 3 mm and dried in a drying cabinet with a temperature of ± 40 °C for a week. The pieces of dried fruit were weighed, mashed, sieved, and a powder was obtained. Add 800 ml of 70% ethanol for maceration. The mass is inserted gradually into the percolator, pour 200 mL of 70% ethanol, filtered with a layer of filter paper, until the liquid drip and is left for 24 hours. Add ethanol repeatedly until you get a layer of filter liquid over the simplicia. The percolates are evaporated with a rotary evaporator at a temperature of not more than 50 ° C

B. Culture of Fusobacterium nucleatum

The T streak technique was employed for culture (T streak). On Chromagar VRE media, *F. nucleatum* was cultured. Markers are used to divide the Petri dishes into three sections. Petri dishes that bacteria have scratched are then tightly closed and incubated for 24 h at 37 ° C in an anaerobic atmosphere, then equalized with McFarland 0.5 or equivalent to a concentration of 1.5x108 CFU/mL

C. Hydrophobicity Assay

The samples were cultured in peptone media, then centrifuged at 2000 rpm for 15 minutes and the supernatant was removed. Each *Fusobacteriumnucleatum* sample was dissolved with PBS pH 7.0, then 100 µl was put in a 96-well microplate. A number of suspensions tested were added with the ethanol extract of Lerak fruit with concentrations (6.25, 12.5, 25) in percent (%).Then incubated for 48 h 37 °C and centrifuged at 2500 rpm for 20 min. The supernatant was removed and 1 ml xylene (Sigma) was added and subsequently placed in a water bath at 37 ° C for 10 min. Vortex for 30 sec to mix the suspension with xylene is stored again in a water bath at 37 ° C for 30 min to separate the suspension and xylene. The precipitate was carefully transferred to another sterile tube, and the remaining xylene in the pipette was resuspended with 2 mL PBS pH 7.0. Then 150 µL of the suspension was added to the 96-well microplate. The hydrophobicity expression of *Fusobacteriumnucleatum* was assessed by optical density at a wavelength of 620 nm utilizing a spectrophotometer (Bio-Rad, USA) (Lee and Lee, 2019).

D. Enzyme Assay

The enzyme approach procedure was started with enzyme extraction by taking one colony of *F. nucleatum*, incubated for 48 h, cultured in BHIB, and the turbidity level is seen after 48 h. Then the Lerak tube in each concentration and incubation time was centrifuged at 3000 g for 30 min to obtain enzymes. The destructive enzyme is part of the precipitate, added with ethanol, centrifuged for 30 min, and put in the freezer for 1 hour. Ammonium sulfate was added, centrifuged for one h, added with 8 M urea in a shaker, left for 15 min, and centrifuged at 3000 g for 30 minutes. Each of the material solutions was put into a 100 µL of the destructive enzyme of *F.nucleatum*based on the concentration into 96 well plates, incubated for 10 minutes, washed with 100 µl of PBS, then incubated for 15 min, washed with PBS 100 µl and in a 100 g, shaker for 2 minutes. I was pipetting the destructive enzyme *F.nucleatum*100 µl to 96 well plates, incubated for 20 minutes, pipetting with anti sera 100 µL, then incubated for 35 min, washed with 100 µl PBS, shaker for 2 minutes, incubated for 20 min, ending with HCL 1 N 50 µl. The results are read with the Elisa reader based on the OD value at a wavelength of 600 nm (Tomás-Cortázar et al., 2018).

E. Dental Root Canal Treatment Modeling

The teeth were made of an outline form to prepare access to the cavity with an endo access bur (Dentsply, Switzerland). Teeth designed for in vitro modeling were then subjected to sterilization. Then 100 µL of BHI medium was inserted into each treatment tooth, incubated for 1.5 hours, then discarded and rinsed with saline. Furthermore, each group injected 25 µl of *F. nucleatum* (1:3) into all treatment groups (25%, 12.5%, and 6.25% of Lerak extract). Furthermore, the teeth inserted with *F. nucleatum* bacteria in the teeth root canal were incubated in an anaerobic incubator for 6 hours. Determination of the working length with the help of a caliper gauge.

Further root canal negotiations with the help of K-file no. 10 (Dentsply, Switzerland). Root canal irrigation using a 5 ml syringe with a two-side vented needle type and a size of 30 G, according to each treatment group. Teeth of all groups were prepared using Mtwo files from files # 10.04 to # 25.06 (VDW, Germany). Irrigation, then dry with paper points. They were subsequently incubated for 24 hours, 48 hours, and 72 hours. After incubation, all the teeth filled with the test material were shaken for 5 minutes at 200 rpm (Siqueira Jr and Rôças, 2022)..

F. Fracture Resistance Test

The sample consisted of 25 mandibular premolar teeth extracted and cleaned. The piece was divided into five groups randomly, each with five models. The sample was placed on an acrylic base block and subjected to a press test (Torsee's Universal Testing Machine). The model was pressed from the occlusal direction of the tooth sample in the order of the tooth axilla (zero degrees). Pressure is constant and slows at a rate of 0.5 mm/min until a fracture occurs. The screen on the machine will show a specific number that states the amount

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of load required to make the tooth break (Uzunoglu-Özyürek et al., 2018).

G. Statistical Analyses

The data obtained from the enzyme destructive test was carried out by the Kruskal Wallis analysis statistical test and other statistics with the Man-Whitney test with a significance level of α = 0.05, while for the hydrophobicity test. The dentin fracture resistance test was carried out as a one-way analysis of the statistical variance test (One Way ANOVA). Further statistics with LSD with a significance level of α = 0.05. At the same time, the correlation value will be analyzed by Spearman and Pearson with r = 1, indicating a strong relationship.

III. RESULTS AND DISCUSSION

Figure 1 shows that the highest value of the anti-hydrophobicity activity of *F. nucleatum* cells is 25% Lerak fruit extract. At the same time, the lowest value was EDTA 17%. In Comparison, the lowest value was EDTA 17%. One way ANOVA statistical test resulted in a p-value <0.05, which indicates that all Lerak fruit extract concentrations have different abilities in inhibiting the hydrophobicity activity of *F. nucleatum*, including other irrigation solutions.

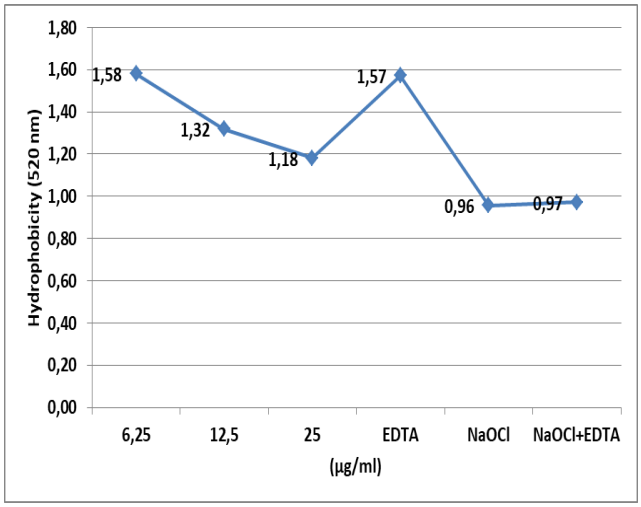

Fig.1: The hydrophobicity activity of *F.nucleatum* influenced by Lerak fruit extrac. The concentration of 25% had better inhibition of hydrophobicity of *F. nucleatum* than other concentrations

Figure 2 showed that the Lerak extract 12,5% has the highest value for reducing the expression of destructive enzymes (p = 0.051). The Kruskal-Wallis statistical test resulted in a p value> 0.05, indicating that all the test material concentrations had the same ability to suppress the expression of the destructive enzyme *F. nucleatum*. The Mann-Whitney test can analyze any differences between each treatment group. The results of the Mann-Whitney test showed that there was no significant difference between each treatment material (p>0,05).

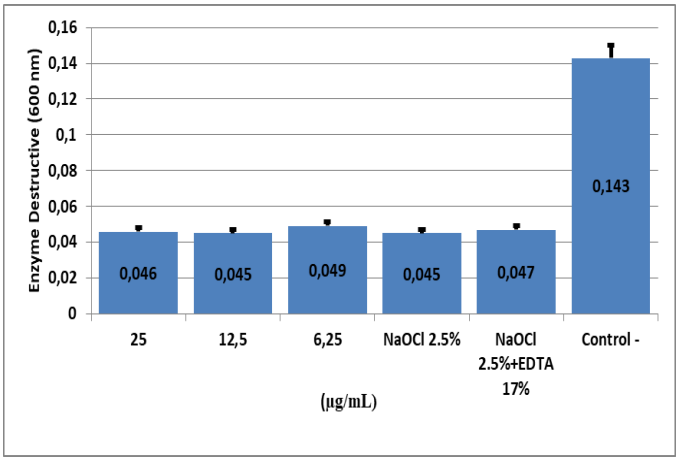

Fig. 2: The destructive enzyme of *F. nucleatum*. Lerak fruit extracts from all concentrations had similar abilities in destroying the *F. nucleatum* enzyme.Bar (Enzyme destruction) Bar Error (Standard deviation)

Figure 3 showed that the concentration at 12.5% generally has better resistance, especially at the 24-hour incubation time. Meanwhile, NaOCl 2.5% + EDTA 17% had a lower fracture resistance value. The concentration of the material does not affect the fracture resistance value (p>0.05). Incubation time also did not affect fracture resistance, but specifically, the two variables showed different effects on each treatment concentration and incubation time. Analysis of the LSD test value of fracture resistance to incubation time showed that there was no significant difference between the incubation time of 24 hours and 48 hours (0.503), 24 hours and 72 hours (0.712), 48 hours and 72 hours (0.760). In Pearson analysis, the concentration and incubation time had no relationship with the fracture resistance value (r = 0.00).

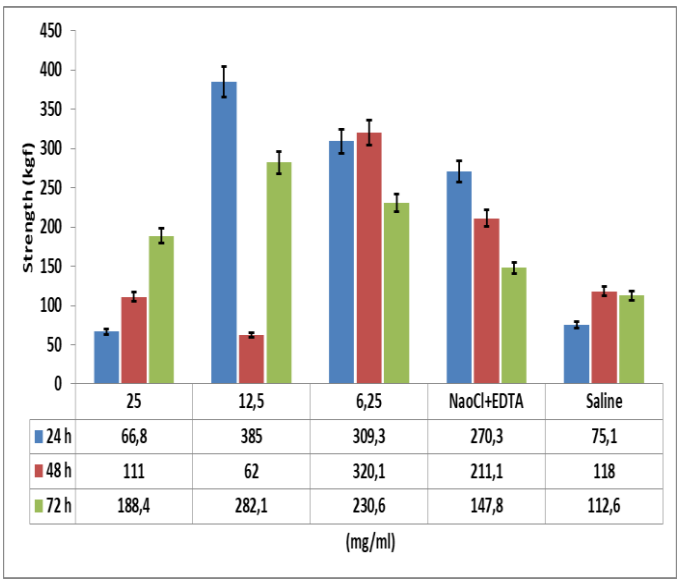

Fig. 3: Fracture resistance of root canal influenced by interaction *F. nucleatum* with Lerak fruit extract. The concentration of 12.5% for 24 hours is higher than the root canal strength value compared to other concentrations

The irrigation material often used in the field of endodontics is sodium hypochlorite. However, NaOCl must be combined with other irrigation materials in the form of chelating materials to remove the inorganic smear layer from the root canals (Raura et al., 2020). EDTA is often combined with NaOCl. However, combining these two materials resulted in increased erosive properties of dentin compared to using the irrigation material alone. Thus, many studies are looking for alternatives to achieve the ideal irrigation solution (Elbahary et al., 2020).

In this study, the ethanol extract of Lerak fruit was used as an alternative for root canal irrigation because it almost met the requirements as an irrigation material. The results related to bioactivity against bacteria in the form of hydrophobicity test showed that the 25% Lerak irrigation solution had a better ability than other concentrations. In the enzyme test results, all concentrations of Lerak fruit extract had the same ability to suppress the expression of the destructive enzyme *F. nucleatum.* The effect of this Lerak extract was presumed because the Lerak extract has many active components. The presence of active components in the Lerak extract irrigation material, such as flavonoids, tannins, and saponins, can affect the structure of the cell surface.

Flavonoids and tannins are phenol derivatives that can interact with proteins, enzymes, and lipids from bacterial cell membranes, thereby changing cell permeability and causing the release of protons, ions, and macromolecules. It decreases bacterial cell surface hydrophobicity (Bilal et al., 2017). The mechanism of action of flavonoid compounds is thought to damage the cell membrane because of its lipophilic nature and ability to form complexes with extracellular proteins. Phenolic compounds are toxic to microorganisms because they can inhibit necessary enzymes, interfering with cell function and damaging protein compounds that can disrupt the semi-permeability of cell membranes (Grgić et al., 2020). Other studies suggest that the mechanism of flavonoids inhibits cell membrane function by disrupting cell membrane permeability and inhibiting the binding of enzymes such as ATPase and phospholipase (Yanto et al., 2020).

Tannins have antibacterial activity related to their ability to activate microbial cell adhesin, activate enzymes, and interfere with protein transport in the inner layer of cells. Tannins also target cell wall polypeptides so that the formation of the cell walls is less than perfect. It causes bacterial cells to become lysed due to osmotic and physical pressure, so the bacterial cells will die (Papuc et al., 2017).

Lerak also contains polyphenols that work through enzyme inhibition by oxidized compounds, possibly through reactions with sulfhydryl groups or nonspecific interactions with microorganism proteins. Polyphenols can also cause the denaturation of bacterial proteins (Papuc et al., 2017).

The results of the fracture resistance test from the LSD test showed that Lerak extract had a significant difference with NaOCl 2.5% + EDTA 17% ($p > 0.05$). This might be due to the mechanism of action of dissolving organic tissue, which is not the same as the complex NaOCl which includes a series of saponification reactions, amino acid neutralization reactions, and chloraminization reactions. The chloraminization response in NaOCl dissolves the dentin organic tissue because the hypochlorous acid in contact with the dentin can dissolve the organic tissue.

The dentin substance will be reduced when NaOCl comes into contact with dentin.[22] EDTA solution can dissolve dentin tissue by reacting with inorganic compounds. Based on the results of Sayin et al. experimental study, using EDTA either alone or before NaOCl resulted in a significant reduction in dentin microhardness. The use of EDTA affects microhardness more than NaOCl. Combining the two further reduces dentin microhardness(Yassen et al., 2015). Meanwhile, Lerak extract can reduce the surface tension by the saponin content and dissolve impurities (debris) due to the surfactants that work in these ingredients.

By the decreased water surface tension, the surface of the water is drawn more firmly to the surface being washed. As a result, the water spreads over the surface of the solid, making it wetter. In dentin, water provides viscoelasticity, increases the ability to absorb stress, and increases the distribution of stress/strain in dentin (Lim et al., 2016).

Hydrated dentin showed higher crack initiation-toughness and crack-growth-toughness compared to dehydrated dentin (Kishen, 2015). Other content in the ethanol extract of Lerak fruit apart from saponins such as flavonoids, polyphenols, and alkaloids has not been known for its cleaning effect on root canal walls.

IV. CONCLUSION

The results of this study have shown that the Lerak extract irrigation material can inhibit the hydrophobicity of *F. nucleatum* and suppress the expression of the destructive enzyme *F. nucleatum.* The fracture resistance test results showed that Lerak extract did not affect dentin fracture resistance.

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