Potential of mangrove (Acanthus Illicifolius) leaf extract as an alternative root canal irrigation in removing smear layer (in-vitro study)

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ABSTRACT
Factor that should be considered in the use of irrigation is the loss of the smear layer on root canal walls. Therefore the prepared root canal can be irrigated using materials, such as EDTA 17% and 2.5% NaOCl and of extract Acanthus ilicifolius 7.5% and 10%. To determine the potential of extract Acanthus ilicifolius leaves as an alternative of root canal irrigation in removing smear layer at a concentration of 7.5% and 10%. The type of this study is a laboratory experimental with post-test only control group design. The total sample is 24 teeth samples that have been prepared and irrigated consisting of four groups. 6 samples for irrigation 7.5% extract Acanthus ilicifolius, 6 samples for irrigation 10% extract Acanthus ilicifolius, 6 positive controls samples for combination 2.5% NaOCl and EDTA 17%, and 6 negative control samples. Shooting tool used to observe the cleanliness level in the walls of the root canal is a Scanning Electron Microscope (SEM). Data were analyzed using Kappa statistics, followed by Kruskal wallis analysis test and continued with Mann-Whitney analysis. Acanthus ilicifolius 7.5% and 10% extracts can remove the smear layer when compared with the negative control group. The combination of 2.5% NaOCl and 17% EDTA is better in smear layer removal compared to 7.5% and 10% Acanthus ilicifolius extracts. Acanthus ilicifolius was able to clean the smear layer on the root canal and can be used as an alternative root canal irrigation

INTRODUCTION
Root canal treatment is the most endodontic treatment performed to maintain teeth in the oral cavity. The success of root canal treatment depends on many factors, including host factors, preparation, microorganisms, etc. As for the 3 main principles in root canal treatment are biomechanical preparation, sterilization and filling of hermetic root canals. The action of root canal preparation which causes friction of endodontic devices with the root canal wall will result in a layer of smear (smear layer) which is attached to the root canal wall and covers the root canal wall. (Farhad et al., 2008; Violich and Chandler, 2010)
Smear layer is a 5-10 μm debris layer formed when the preparation of a tooth root canal is composed of organic and inorganic components from calcified tissue, necrotic tissue, odontoblasts and other microorganisms including denatured hydroxyapatite and collagen. Smear layer has an amorphous structure and is in the form of granules that cover the root canal wall to the dentinal tubules found in the root canal after preparation. (Eve et al., 2015; Zakarea et al., 2014)

The presence of a smear layer can cause the failure of root canal treatment. The bacteria may be left behind on the smear layer even after chemomechanical preparation. The smear layer also interferes with the physical characteristics, quality, adaptation and attachment of material into the root canal wall if not eliminated. (Zehnder, 2006)

Removal of the smear layer is an important consideration in root canal treatment. Mechanical instrumentation measures must always be accompanied by root canal irrigation with the use of irrigation materials that can get rid of the smear layer. However, no single irrigation material can simultaneously have the ability to removing of overall smear layer. Root canal irrigation is an important step in supporting the success of root canal treatment because irrigation facilitates the removal of necrotic tissue, microorganisms and dentine splinters from infected root canals with the action of irrigation solution rinse. (Carver et al., 2007)

Root canal irrigation materials that can be used include 2.5% Sodium hypochlorite (NaOCl) and Ethylenediaminetetraacetic Acid (EDTA) 17%. (Munley and Goodell, 2007) Sodium hypochlorite is one of the effective irrigation ingredients to dissolve the remaining pulp tissue and organic dentine and has an antimicrobial effect. The usual concentration of sodium hypochlorite is 0.25% - 6%. Several studies suggest that sodium hypochlorite effectively inhibits Enterococcus faecalis bacteria when compared to other irrigation materials

Besides its effectiveness, Sodium hypochlorite has toxicity, which can cause severe irritation and inflammation around the tissue if material extrusion occurs during irrigation. EDTA (Ethylenediamine-tetraacetic Acid) is an irrigation material that can dissolve inorganic components from the smear layer. But denaturation on collagen fibers can interfere with the adaptation of sealer to the dentin walls of root canals. EDTA (chelating agent) has a weak antibacterial effect, to get better results, this solution must be combined with other irrigation materials that are capable of dissolving organic tissue and a fairly strong antibacterial effect.

Calc and Serper reported that the use of 17% EDTA for 10 minutes as irrigation material could cause erosion of the peritubular and intertubular dentin because EDTA uses affected dentin micro roughness. (Silveira et al., 2013; Pashley et al., 2002), evaluated cytotoxic of 17% EDTA solution with a citric acid solution to cultured fibroblasts. The results showed that EDTA solution 17% had a higher toxic effect than the citric acid solution. EDTA is also an imported irrigation material that has an uneconomical price of. (Metzger et al., 2011; Beltz et al., 2003)

The field of dentistry many have used natural ingredients as clinical and laboratory materials or as alternative ingredients, one of which is mangrove (Acanthus ilicifolius) leaves. According to research that has been done on the chemical composition of Acanthus ilicifolius in the health field as neuralgia, analgesic, anti-inflammatory, antioxidant, antifertility, hepatoprotective, antitumor, anti-leukemia, anticancer, antimicrobial, antiviral and antifungal as artificial insecticides can be used. (Irawanto, 2014)

According to previous research, Acanthus ilicifolius is rich in saponin compounds, flavonoids, steroids, alkaloids, terpenoids, and tannins. Saponins, alkaloids, terpenoids and tannins have the ability to interfere with the integrity of bacterial cell membranes so that they can cause lysis of bacterial cells. Saponin is also an emulsifier (detergent) which can dissolve organic and non-organic smear layers and reduce surface tension and increase dentin permeability which facilitates penetrating adhesive materials. (Govindasamy and Arulpriya, 2013; Khajure and Rathod, 2010)

MATERIALS AND METHODS

This research is a laboratory experimental study with a post-test only control group design. 24 extracted mandibular premolars were grouped into 4 treatment groups. 6 samples were irrigated with 7.5% Acanthus ilicifolius leaf extract, 6 samples were irrigated with 10% Acanthus ilicifolius leaf extract. 6 samples were irrigated with a combination of 2.5% NaOCl and EDTA 17%, and 6 samples were irrigated with saline solution as a negative control.

The sample used in this study was mandibular premolar which had been extracted and stored in saline solution, with the criteria that the root of the tooth was still intact, the root canals were wide and straight, the roots were not carious. The dental crown was cut to the extent of the cementoenamel junction, working length of the entire sample was determined by measuring the tooth length and minus 1 mm. Root canal irrigation using a 3 ml syringe with a type of two-side vented needle mea-
The application of this irrigation technique with irrigation needles is bent, and the position of the needle should be loosely inside the root canal in order for reflux to occur from the irrigation material, and the debris will be carried to the coronal root canal. Length of needle penetration recommended is 1 mm of working length.

All samples were carried out root canal preparation with a crown-down pressureless technique using the Universal ProTaper NiTi Sx rotary till up to file F3. Down without pressure begins with the largest file Sx / Gates Gliden Drill 1/3 coronal preparation. Determine the working length by using K-file # 15, which can be used as a reference for preparation with S1 and S2 files.

Root canals were irrigated by moving the disposable syringe with irrigation solution, i.e., 6 samples were irrigated with *Acanthus ilicifolius* extract 7.5%, 6 samples were irrigated with *Acanthus ilicifolius* extract 10%, 6 samples were irrigated with a combination of 2.5% NaOCl and EDTA 17%, and 6 the sample was irrigated with normal saline. Irrigation is done at every file change. Then, in the same way, proceed with preparation with S1 file, S2 with brushing motion technique. Irrigation, recapitulation and re-preparation were performed with finishing files namely, F1, F2, F3 with up and down motion and final irrigation and dry using paper points.

After irrigation, the root canals were dried with paper points. Then each sample was measured from the cementoenamel junction from the buccal / lingual direction to the end of the apex by using the term, and the ruler then marked with a black marker. Samples that are given a mark will bur by separating disk and splitting using a chisel.

To evaluate the results of cleanliness, all samples were investigated by Scanning Electron Microscope (SEM). The assessment was carried out using a transparent sheet tool cut according to the size of the photo into 9 boxes by attaching the assessment score. The assessment was carried out by 2 observers with a double-blind method where the scores were as follows:

Score 1: No smear layer, dentinal tubuli open.
Score 2: Small amount of smear layer, some dentinal tubuli open.
Score 3: Homogenous smear layer covering the root canal wall,
only a few dentinal tubuli open.
Score 4: Complete root canal wall covered by a homogenous smear layer, no open dentinal tubuli.
Score 5: Heavy, nonhomogenous smear layer covering the complete root canal wall. (Zehnder, 2006)

RESULTS AND DISCUSSION

Based on the research that has been done, the results of the research are as follows:

![Figure 1: SEM image of group irrigated with Acanthus ilicifolius extract 7.5%](image1)

![Figure 2: SEM image of group irrigated with Acanthus ilicifolius extract 10%](image2)

![Figure 3: SEM image of the group with a combination of 2.5% NaOCl and EDTA 17%](image3)
Based on the description of Scanning Electron Microscope, scoring of the smear layer is carried out in the root canal by two observers. Then the analysis is done using the Kappa statistic to see whether there is a difference between observer 1 and observer 2.

The results of the statistical kappa test obtained a kappa value = 0.91 (P>0.05), which indicates there was no difference in the observation of the score between observers 1 and 2. The results of the observer 1 scores were taken from each treatment group, then Kruskal Wallis analysis to see if there were significant differences between all groups treated the tooth root canal smear.

From the results of the analysis of kruskal wallis obtained p-value <0.05 (p = 0.000) which shows that there is a difference in the effect of irrigation from Acanthus ilicifolius extract 7.5%, Acanthus ilicifolius extract 10%, 2.5% NaOCl solution and EDTA 17% and 0.9% saline solution to the tooth root canal smear.

To see each difference between each treatment group, the Mann-Whitney analysis test was used.

Based on the research, the results of the scanning electron microscope showed that the solution of Acanthus ilicifolius extract 7.5% and 10% (Figure 1 & Figure 2) had been able to remove the smear layer on the apical third of the root canal when compared with the negative control, i.e. 0.9%. This can be seen from the results of the Mann-Whitney test where it is known that p <0.05, which is a difference in cleaning the smear layer on the root canal. (Table 1) This occurs because the ability of Acanthus ilicifolius extract in removing the smear layer is due to the presence of saponin which is an active component of Acanthus ilicifolius extract which acts as a surfactant or detergent which can reduce surface tension. (Govindasamy and Arulpriya, 2013)

Saponin consist of hydrophilic groups and hydrophobic groups where hydrophilic groups will bind to polar compounds (organic smear layer) and hydrophobic groups will bind to non-polar compounds (inorganic smear layer). In addition to reducing surface tension, saponin can also increase penetration of irrigation solutions until to the apical third of the root canal in the main root canal and also in the root canal area which cannot be reached by instrumentation such as areas of root canal ramification and increasing contact with irrigation material with the root canal wall. (Mancini et al., 2013; Khasanah, 2009)

From the comparison of Acanthus ilicifolius extract with the positive control group, namely the combination of 2.5% NaOCl and 17% EDTA, it was found that there was a significant difference in the Mann-Whitney analysis, that the combination of 2.5% NaOCl and EDTA was 17% better than extract Acanthus ilicifolius extract 7.5% and 10% in removing the smear layer. (Figure 3)

This can be seen from the use of 2.5% NaOCl irrigation in combination with EDTA 17% often used as root canal irrigation material to get the effect of removing organic and inorganic smear layers. It was seen that the combination of 2.5% NaOCl and 17% EDTA was the best material in removing the smear layer on the root canal of the tooth which is the Gold Standard. (Vineet et al., 2014)

This is in accordance with the research of (Silveira et al., 2013) reported that a combination of 2.5% NaOCl and 17% EDTA even though it was proven effective in removing organic and inorganic smear layers in the coronal third and middle third part of the root canal, but ineffective the apical third of the root canal. Dissolving the smear layer on the apical third is associated with anatomic variations in the root canal, especially in the apical part of the root canal. (Silveira et al., 2013)

The ability of the irrigation material to remove the smear layer studied in the scanning electron microscope with 1000X magnification shows that Acanthus ilicifolius extract 7.5% and 10% have been able to remove the smear layer on the apical third of the root canal when compared to the control group, i.e. 0.9% saline. (Figure 4) In this study, there were obstacles with the use of 7.5% and 10% Acanthus ilicifolius extract as irrigation material. The irrigation materials of Acanthus ilicifolius extract 7.5% and 10% have not been as effective as the combination ingredients of 2.5% NaOCl and EDTA 17%. It is possible that the irrigation solution of Acanthus ilicifolius extract has a saponin content which is slightly at concentrations of 7.5% and 10%, so it needs to
be investigated further the appropriate concentration in removing the smear layer at concentrations higher than 10%. This is consistent with previous studies using natural ingredients as root canal irrigation materials, which use higher concentrations of 100%.

**CONCLUSIONS**

Based on the results of the study, it can be concluded that *Acanthus ilicifolius* mangrove leaf extract at a concentration of 7.5% and 10% as well as 2.5% NaOCl and 17% EDTA combination based on statistical test results (P < 0.002) have significant differences among the groups. Based on the statistical test results it was found that 2.5% NaOCl and 17% EDTA combination is better in removing the Smear layer than the *Acanthus ilicifolius* extract 7.5% and 10% but *Acanthus ilicifolius* extract 7.5% and 10% have better result than saline material in the field of dentistry in cleaning the smear layer.

**REFERENCES**


