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Authors: Tamara Yuanita**, Uli Sasi Andari*, Mandojo Rukmo**, Sukaton**, Deavita Dinari*

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Research Report

Contrasting efficacy of cocoa POD HUSK extract and 8% propolis extract in maintaining of root canal wall cleanliness

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ABSTRACT

Background: *The existence of the smear layer, which can be produced during root canal instrumentation, may compromise the bond between filling material and the root canal walls. Therefore, the use of an effective root canal irrigation solution, a commonly employed form of which is sodium hypochloride (NaOCl), is important. Sodium hypochloride has several positive properties including effectiveness as a disinfectant agent and its ability to promote tissue-dissolution, although it is ineffective at cleaning the smear layer. There have been numerous recent studies of the application of phytomedicines in endodontics due to their advantages such as minimum toxicity and cost effectiveness. The saponin contained in both the propolis and cocoa pod husk acts as a surfactant that may lower surface tension and dissolve debris containing organic and anorganic materials.* ***Purpose:*** *The study aimed to provide evidence of the differences between root canal wall cleanliness when treated with 8% propolis extract and different concentrations of cocoa pod husk extract.* ***Methods:*** *25 extracted teeth with single straight root canals were randomly divided into five categories (n=5). Sample preparation was performed using a rotary file and irrigated with different solutions. The first group was administered 2.5% NaOCl, the second group 8% propolis, the third group 3.12% cocoa pod husk extract, the fourth group 6.25% cocoa pod husk extract, and the fifth group 12.50% cocoa pod husk extract. The samples were then dissected into two sections at the apical third and their cleanliness scores subjected to a Mann-Whitney test with a significance level of p=0.05.* ***Results:*** *A significant difference was identified between all groups (p<0.05) and on the median control test, the highest value of 1.6 was recorded by the 6.25% cocoa pod husk extract, compared to the other four groups* ***Conclusion:*** *Cocoa pod husk extract demonstrates greater efficacy at cleaning root canal walls compared to 8% propolis extract.*

Keywords: *cocoa pod husk extract; propolis extract; root canal irrigation*

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INTRODUCTION

The main purpose of root canal treatment is to disinfect the entire canal by eliminating microorganisms and other microbe components in order to prevent reinfection both during and after the treatment. Chemo-mechanical debridement can achieve this objective during root canal treatment.[1] The debridement process includes cleaning and shaping of the canal to remove necrotic tissue residue, bacteria and the smear layer in order to facilitate sterilization and obturation of the canal.[2] Chemical debridement is important for teeth with a challenging anatomy, such as fins or other irregularities, that might be undetected by instrumentation.[3] The ideal properties of irrigation solution comprise: a broad antimicrobial spectrum, low toxicity, the ability to solve the problems of necrotic tissue and debris, and low surface tension, while also being capable of dissolving the smear layer.[4] Sodium hypochlorite (NaOCl) is a commonly used irrigation solution, mainly because of the fact that it constitutes a cheap antiseptic lubricant possessing the ability to dissolve necrotic materials. The major disadvantages of NaOCl are its cytotoxicity on

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entering the periradicular tissue and its inability to dissolve the smear layer. Moreover, both its odor and the taste are unpleasant.[5]

A considerable amount of research into natural ingredients as an alternative to conventional dentistry materials has recently been undertaken because of their advantages compared to commonly used chemical materials, including: widespread availability, cost effectiveness, low toxicity, and also their lower susceptibility to microbial resistance.[6] One natural ingredient commonly used for research purposes is propolis, a bee-produced material containing a mixed complex consisting of wax, a small amount of sugar, and tree sap collected by honey bees *(Apis mellifera)*.[7] At a concentration of 8%, propolis is more effective at cleaning the root canal walls of smear layer compared to 2.5% NaOCl.[8]

In addition to propolis, another natural ingredient whose use is rapidly gaining in popularity is cocoa pod husk extract *(Theobroma cacao)* which contains in excess of 500 different chemicals and has traditionally been used as an antioxidant, anticarcinogenic, immunomodulator, vasodilator, antimicrobial, and analgesic.[9] Cocoa pod husk extract can impede the formation of *Enterococcus faecalis* (*E. faecalis*) bacterial biofilm at a minimum biofilm inhibitory concentration (MBIC) of 3.12% and possesses significant potential as an alternative root canal irrigation agent. The other concentrations of 6.25% and 12.50% are selected because the higher the concentration, the lower the optical density value of biofilm.[10] This research was undertaken in order to obtain knowledge about the relative cleaning effectiveness of cocoa pod husk extract and extract of 8% propolis in relation to the root canal walls.

MATERIALS AND METHODS

The experiment employed 25 human mandible premolar samples, previously extracted for orthodontic reasons, which satisfied the following criteria: single canal teeth with an average length of 20 ± 2 mm; a post-access gauging process using a NiTi file #8, #10, or maximum #20; a good fit at the apex, and defect-free closure of the apical foramen.

The mandible premolar teeth meeting the criteria were soaked in saline and divided into five groups, each containing five teeth. Access opening was completed by means of a high-speed endo access bur. Files no. 8 to 10 were used to determine the working length of each sample, supported by a gauging process. Preparation of the root canal involved the use of rotary files (Protapper NEXT, Dentsply Sirona, Tulsa) combined with an endomotor (X-smart plus, Destsply Sirona, Tulsa). Each file preparation took approximately ten seconds. During file exchange, the irrigation solution was divided as follows: the first group was supplied with 2.5% NaOCl, the second group 8% propolis extract, the third group 3.12% cocoa pod husk extract, the fourth group 6.25% cocoa pod husk extract, and the fifth group 12.50% cocoa pod husk extract.

The irrigation process was performed with an instrument set up in such a manner that the air pressure was at 1 atm (1033kg/cm^2). The 3ml of irrigation solution were applied for ten seconds on each occasion. Therefore, the total amount of irrigation solution for each sample was 12 ml. During the final irrigation process, the canal was activated by means of EDDY (VDW, Germany) before, finally, being dried with sterile paper points and closed with temporary restoration.

All samples from each group were marked on the lingual and buccal side using a high-speed diamond disc as a cutting guide, prior to being cut horizontally through the apical third of the tooth (4mm from the apex) by means of a disc bur. The samples were then bisected with a chisel and affixed to a sample holder (stub) with the surface of the root canal upward facing using a specific glue (Araldite®, Switzerland) that had been mixed with aluminium powder. Having been allowed to air dry for a day, the surfaces of the samples were coated for approximately one hour with pure gold or carbon for later observation with a vacuum evaporator. At that point, the samples were ready for observation by means of a scanning electron microscope (SEM).

The samples were individually inserted into the SEM for observation of their middle sections at a magnification of 150x. This section was subsequently magnified again at 1000x, having been set up for the particular contrast and lighting. Evaluation of the photomicrograph was completed by two observers. The field of view was divided into nine boxes identical in size (three cubes).[11]

Evaluation of the SEM image was conducted with the following scoring system for each box:[6] score 1 indicated the absence of a smear layer and that dentinal tubule surfaces were clean, score 2 signified that 25% of the root canal wall surface was covered by a smear layer, score 3 denoted that 25% to 50% of the root canal wall surface was covered by a smear layer, score 4 showed that 50% to 75% of the root canal wall surface was covered by a smear layer, score 5 indicated that more than 75% of the root canal wall surface was covered by a smear layer. A non-parametric Kruskal-Wallsis test was performed to establish the difference for all groups followed by a Mann-Whitney test to identify the difference between each group with a p-value lower than 0.05. In such cases, the difference was considered to be significant.

RESULTS

The cleanliness scores recorded 25 samples of teeth divided into five groups are shown in Table 1. From the statistical analysis, the average cleanliness score of the first group was the lowest, indicating that it contained a smear layer covering between 50% and 75% of the root canal walls. In contrast, the highest cleanliness score was recorded by the

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fourth group signifying the absence of a smear layer and clean dentinal tubules. Figure 1 contains the SEM image of each group which shows the cleanliness of root canal walls free of smear layer.

The result of a Kruskal-Wallis test indicated that the significance level was 0.000 (p-value<0.005) meaning that a significant difference existed between all groups. The Mann-Whitney test identified the differences between each group and the results contained in Table 2 show how the groups compare to each other. All of the numbers represent a value lower than 0.05 which indicates that significant data discrepancies existed between each group.

Table 1. Cleanliness score for each group

Group	Cleanliness score
2.5% NaOCl	4.7
8% Propolis	3.4
3.12% Cocoa	5
6.25% Cocoa	1.6
12.50% Cocoa	2.5

DISCUSSION

Root canal cleanliness constitutes one of the parameters for effective root canal treatment since research has shown that the smear layer on the root canal walls covers the dentinal tubules. A smear layer constitutes a mixture of organic and inorganic particles that can accumulate during the preparation process relating to the root canal walls[8] and whose cleanliness is observable under SEM, thereby enabling calculation of the number of clean and unclogged dentinal tubules.[11,12] A significant body of recent research has been undertaken into the natural ingredient phytomedicine because of its advantages when compared to the chemical agents currently widely employed within the field of dentistry.[6]

From statistical analysis data, the average cleanliness score of the control group which uses NaOCl as irrigating solution was considered to be high. This was due to the inability of NaOCl to dissolve the inorganic substance and its high surface tension which render it more difficult to clean and disinfect the entire root canal system with the result that the smear layer covers the root canal walls.[13] The

Table 2. Results of differences between group

Group	2.5% NaoCl	8% Propolis	3.12% Cocoa	6.25% Cocoa	12.50% Cocoa
2.5% NaOCl					
8% Propolis	0.009*				
3.12 % Cocoa	0.044*	0.005*			
6.25 % Cocoa	0.009*	0.009*	0.005*		
12.50 % Cocoa	0.009*	0.036*	0.005*	0.009*	

* p-value < 0.05 means the the data has a significant data discrepancy.

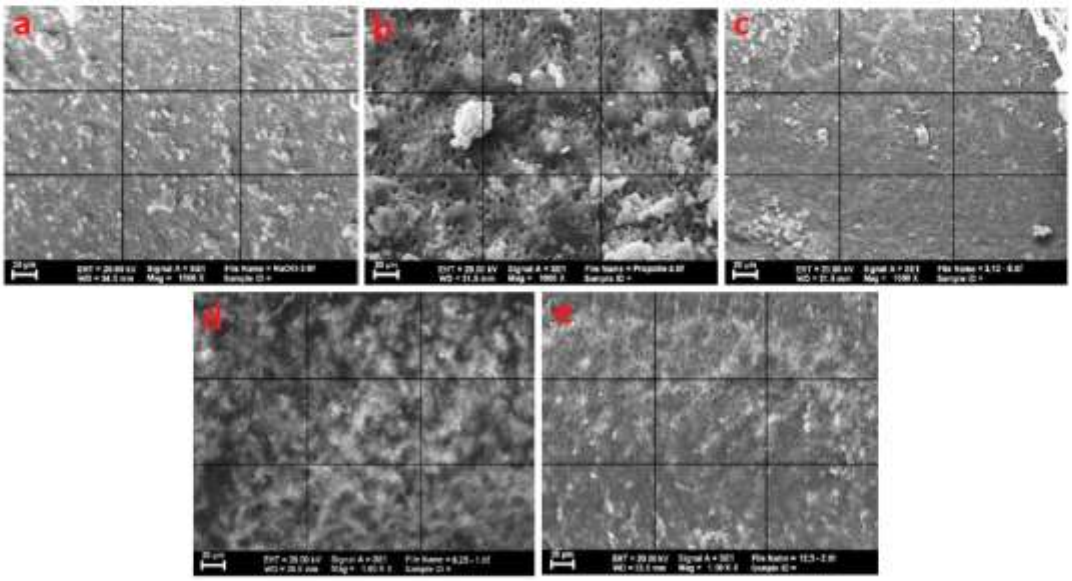

Figure 1. (a) SEM image for control group 2.5% NaOCl; (b) SEM image for 8% propolis; (c) SEM image for 3.12% cocoa; (d) SEM image for 6.25% cocoa; (e) SEM image for 12.50% cocoa.

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8% propolis extract produced an average cleanliness result and in comparison to 3.12% cocoa pod husk extract was more effective at cleaning the root canal walls. However, the result was different when compared to 6.25% and 12.50% cocoa pod husk extract because both concentrations are more effective at cleaning the root canal walls. Both extract of propolis and cocoa pod husk contain saponin, although at differing concentrations. According to the examination results produced by the Surabaya Research Center and Industry Consultant Laboratory the concentration of saponin in the propolis extract is lower (0.88%) than that of cocoa pod husk extract (2.18%). Consequently, the efficacy of saponin in the propolis is reduced.

The extract of 3.12% cocoa pod husk recorded the highest root canal wall cleanliness score indicating that it had not yet reached the critical micelle concentration (CMC) at which point a surfactant initiates the formation of micell capable of dissolving fat and oil. This means that at a concentration of 3.12%, the amount of saponin in cocoa pod husk extract was insufficiently effective to clean the smear layer from root canal walls. 6.25% cocoa pod husk extract recorded the lowest cleanliness score among all the other treated groups. This signified that a concentration of 6.25% was the most effective at cleaning the smear layer from root canal walls compared to other treatment groups. Based on these results, once that concentration of CMC had been reached the micelle started to form and was able to clean inorganic material and smear layer from the the root canal walls.

According to the statistical analysis results, a significant difference existed between the fourth and fifth groups, where the average score of the fourth group was lower, which means that at a concentration of 6.25% the cocoa pod husk extract was more effective than at one of 12.50%. This possibly occurred because of the theobromin content demonstrating an ability to increase enamel hardness by substituting for the hydroxyapatite crystal lost through the demineralisation process. For example, since theobromin crystal size is smaller than that of hydroxyapatite, this facilitates its penetrating the microtunnel of enamel or dentin and exchanging the apatite ions. This explains why cocoa pod husk extract (12.5%) had a lower cleanliness score at higher concentrations than 6.25% cococa pod husk extract.[14,15] Therefore, the higher concentration of cocoa pod husk did not invariably induce efficacy of cleanliness in root canal walls due to its theobromin content. In conclusion, the efficacy of root canal wall cleanliness differed between 8% propolis extract and cocoa pod husk extract. Cocoa pod husk at 6.25% demonstrated the greatest efficacy in promoting root canal wall cleanliness compared to 8% propolis extract.

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