

## Research Report

## The effective concentration of red betel leaf (*Piper crocatum*) infusion as root canal irrigant solution

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### ABSTRACT

**Background:** Smear layer is a debris consisting of organic and inorganic particles of calcified tissue, necrotic tissue, pulp tissue, and dentinoblast and microorganism processes that can close the entrance to the dentin tubuli. Smear layer, will not only inhibit the penetration of disinfection materials and sealers to the dentin tubuli, but will also reduce the attachment of root canal filling material so that root canal irrigation solution is needed to dissolve the smear layer. Red betel leaf (*Piper crocatum*) infusion, on the other hand, contains saponin characterized as "surfactants" which can dissolve smear layer. Nevertheless, the effective concentration of the red betel leaf infusion has still not been known clearly. **Purpose:** This study is aimed to determine the effective concentration of the red betel leaf infusion for cleaning root canal walls from smear layer. **Methods:** Fifteen extracted human teeth with straight single roots were randomized into 5 groups (n=3). The specimens were then shaped by using rotary instruments up to a size of 25/07. During instrumentation, each canal was irrigated with 10, 20, 30 and, 40% red betel leaf infusion for treatment groups, while another was irrigated with aquadest for the control group. Root canal cleanliness was observed by using scanning electron microscope (SEM). **Results:** There were significant differences among treatment groups ( $p < 0.05$ ), except in the treatment groups irrigated with red betel leaf infusion with concentrations of 30% and 40% ( $p > 0.05$ ). **Conclusion:** It can be concluded that red betel leaf infusion with a concentration of 30% is effective for cleaning the root canal walls from the smear layer.

**Key words:** Red betel leaf infusion (*Piper crocatum*), smear layer, saponin, surfactant

### ABSTRAK

**Latar belakang:** Smear layer adalah suatu debris yang mengandung partikel organik dan anorganik dari jaringan terkalsifikasi, jaringan nekrotik, proses dentinoblas, jaringan pulpa dan mikroorganisme yang dapat menutup jalan masuk ke tubuli dentin. Smear layer akan menghalangi penetrasi dari bahan disinfeksi dan sealer terhadap tubuli dentin dan mengurangi perlekatan bahan pengisi saluran akar, sehingga dibutuhkan larutan irigasi yang dapat membuang smear layer tersebut. Infusa daun sirih merah (*Piper crocatum*) mengandung saponin yang dikarakteristikan sebagai surfaktan yang dapat melarutkan smear layer, tetapi sampai sekarang belum ada penelitian tentang hal tersebut. **Tujuan:** Penelitian ini dilakukan untuk mengetahui konsentrasi efektif daya pembersih infusa daun sirih merah (*Piper crocatum*) dapat membersihkan dinding saluran akar dari smear layer. **Metode:** 15 gigi premolar bawah manusia yang mempunyai akar lurus dibagi menjadi 5 kelompok secara acak (n=3). Gigi dipreparasi menggunakan rotary instrumen sampai Protaper F2 (30/0.02). Selama instrumentasi, dilakukan irigasi dengan infusa daun sirih merah (*Piper crocatum*) dan konsentrasi 10, 20, 30, 40% dan diirigasi aquadest untuk grup kontrol. Setelah itu kebersihan dinding saluran akar diperiksa dengan menggunakan scanning electron microscope (SEM). **Hasil:** Terdapat perbedaan yang signifikan antara masing-masing kelompok ( $p < 0,05$ ), kecuali kelompok yang diirigasi infusa daun sirih merah (*Piper crocatum*) konsentrasi 30% dan 40% ( $p > 0,05$ ). **Kesimpulan:** Dapat disimpulkan infusa daun sirih merah (*Piper crocatum*) dengan konsentrasi 30% efektif untuk membersihkan dinding saluran akar dari smear layer.

**Kata kunci:** Infusum daun sirih merah (*Piper crocatum*), smear layer, saponin, surfaktan

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## INTRODUCTION

Endodontic treatment is actually considered as an effort to maintain teeth as long as possible in oral cavity by taking the entire pulp tissue, either from pulp chamber or root canal.<sup>1-3</sup> The principles of endodontic treatment, moreover, involves preparation, sterilization and filling root canals. The preparation of root canal can be considered as one of the important stages in the endodontic treatment.<sup>1</sup> This stage involves smear layer cleaning and root canal shaping, so it can facilitate sterilization and filling the root canal.<sup>1,4</sup> Smear layer can be defined as a debris composition consisting of organic and inorganic particles of calcified tissue, necrotic tissue, and odontoblast and microorganism processes. Smear layer, therefore, can contain bacteria, and this layer even can close the entrance to the dentine tubuli so that it will reduce the attachment of root canal filling material.<sup>4-6</sup>

During and after the preparation stage, or every changing of preparation number, the irrigation of root canal should always be conducted. In general, the term of irrigation means watering, washing, or cleaning by using irrigation liquid.<sup>3,5</sup> This root canal irrigation process is aimed to remove necrotic tissue, to water root canals, and to dispose smear layer, so it can simplify the implementation of the preparation as well as can reduce the number of microorganisms in root canals.<sup>7-11</sup>

Red betel leaf (*Piper crocartum*) is one of the natural materials that could potentially be used as an ingredient of root canal irrigation.<sup>5,9,11</sup> Red betel leaf contains saponin compound characterized as "surfactants" which can lower the surface tension so that it has an ability to dissolve organic materials, inorganic materials, and microorganisms in root canal.<sup>12,13</sup> As root canal irrigation material, red betel leaf infusion must have a certain concentration, but until now there has been no research on it.<sup>15-17</sup> Thus, it is necessary to find the effective concentration of red betel leaf infusion used as a biocompatible root canal irrigation solution.<sup>18-20</sup> The purpose of this research is to determine the effective concentration of red betel leaf infusion for cleaning the root canal walls of smear layer.

## MATERIALS AND METHODS

Fifteen lower premolars that had been extracted for orthodontic purposes were immersed in normal saline solution, and then classified into five groups, each group consisted of three teeth. Dental radiography concerned with buccal and mesial aspects was then conducted on those

teeth in order to ensure closed apical. Both of the thickness of the root canal, especially in one third of the apical, and the length were confirmed by radiographic images using a file with a size of 20/.07. The preparation of root canal was then conducted with rotary protaper until protaper F2 (25/.07) with the preparation duration of 10 seconds for every protaper.

During the changing of the instruments, the irrigation of the root canal was conducted based on the irrigation solution used. Group 1 using 10% red betel leaf; group 2 using 20% red betel leaf; group 3 using 30% red betel leaf; group 4 using 40% red betel leaf; and group 5 using aquadest as control. These irrigation processes were conducted by using a tool that had been prepared so that the air pressure became the same, about 50 mpa/kg. The irrigation solution used for each irrigation process was about 3 ml for 5 seconds, so the total amount of red betel leaf infusion used for each tooth sample was about 15 ml. After the irrigation processes, the root canal was dried with sterile paper for three times.

Afterwards, those samples were cut horizontally at one third of the apical, about 4 mm from the edge of stale, by using disc bur (save side disk). Then, they were cut longitudinally with a chisel in order to become two parts with the same size. Those samples cut already were planted or placed on sample holder with root canal surface facing up. Then, it was inserted into air barrier tool from coating unit. Coating process with gold was conducted for 40 seconds, and then was ready to be examined by scanning electron microscopy (SEM). At first, the entire the apical region was observed with 150x magnification in the middle. Later, this area was enlarged to the size of 1500 which contrast and brightness were set. By pressing a certain button, one third of the area apical could also be observed.

The amount smear layer of each specimen were scored by four blinded evaluator. The scores were determined as follows (5): 1 = There was no smear layer, and all dentin tubuli were open and clean; 2 = There were few smear layers, and some dentin tubuli were open and clean; 3 = There was homogeneous smear layer covering some root canal walls; 4 = There was homogeneous smear layer covering the entire root canal walls; 5 = There was non-homogeneous smear layer covering the entire root canal walls (heavy).

Cohen's Kappa statistical analysis was used to analyze agreement among the evaluator. The differences within each group were then analyzed by using non-parametric analysis of Kruskal-Wallis test. And, Mann-Whitney test was finally conducted followed with control median test.

## RESULTS

The absence and presence of smear layer are presented on figure 1. Based on Cohen Kappa's test, values obtained was above 0.07 indicating a good grade in which there was the same perception of the valuation methods among the observers. The assessment results of the root canal cleaning power of red betel leaf infusion at concentrations of 10%, 20%, 30%, and 40%, and that of aquadest as in the control group can be seen in table 1.

**Table 1.** The mean and standard deviation of the smear layer in each group

Groups	Mean	Standard Deviation
10%	3.44	0.52
20%	1.88	0.60
30%	1.22	0.44
40%	1.22	0.44
Aquadest	4.66	0.50

In this research, several variables were controled in order to obtain homogeneous samples. Non-parametric test was conducted with the Kruskal-Wallis test to determine whether there was any difference among all of those treatment groups. Based on the results of the test, it is then known that the significance level was about 0.001, smaller than 0.05 ( $p < 0.05$ ). This indicates that there were significant differences in all treatment groups.

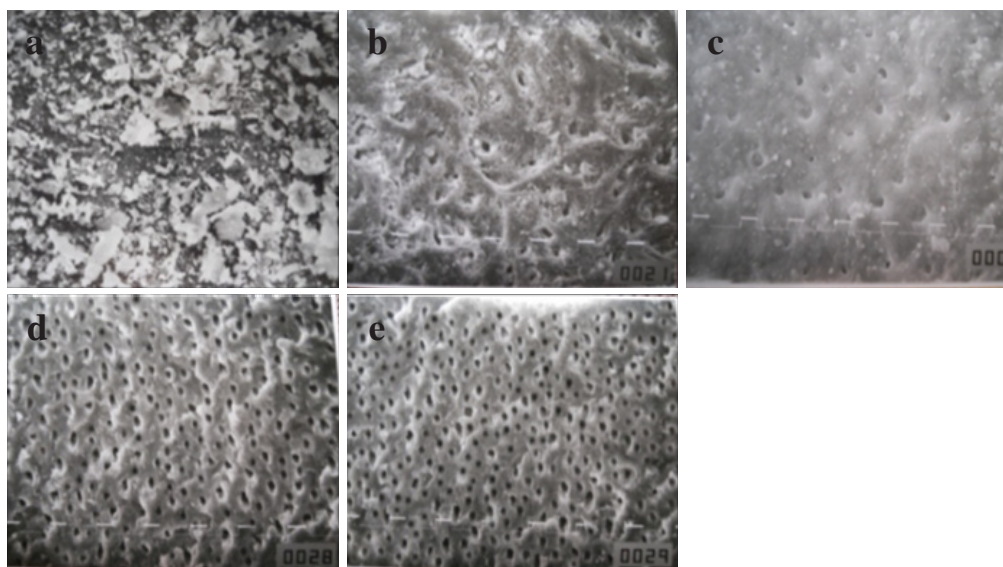
Afterwards, Mann-Whitney test was conducted to determine the differences in each of those treatment groups.

Based on the results of the test, it is then known that the significance level of in each of those treatment groups was about 1.000, ( $p > 0.05$ ). This indicates that there was no significant difference in those treatment groups. Control Median test was conducted to find out the median of each group. Based on the results of the test, it is then known that median value of red betel leaf infusion with a concentration of 30% was the same as that of red betel leaf infusion with a concentration of 40%, about 1.000, considered as the smallest value compared with the other groups. This suggests that red betel leaf infusion with a concentration of 30% was the most effective value.

## DISCUSSION

At the preparation stage, during and after the stage, or every changing of file number, the irrigation of root canal should always be conducted, therefore, the use of irrigation solution during the preparation is important.<sup>2-4</sup> Smear layer is used as an indicator of the level of cleanliness because every act of root canal preparation will occur friction endodontic tool with root canal wall resulting in the formation of a layer of debris attached to the walls of the root canal known as smear layer.<sup>5,26,27</sup>

The effective concentration of red betel leaf infusion can be known by conducting laboratory research and then by using SEM showing the absence of smear layer and all the open and clean dentine tubuli. Thus, the more open dentin tubuli is, the more clean root canal walls of the smear layer.<sup>21-23</sup> SEM was chosen because this tool can be



**Figure 1.** SEM photograph of the surface rootcanals in group: a) control: the entire root canal walls were covered with non-homogeneous smear layer (heavy), (b) 10% red betel leaf infusion: the entire root canal walls were covered with homogeneous smear layers, (c) 20% red betel leaf infusion: the entire root canal walls were covered with homogeneous smear layers, (d) 30% red betel leaf infusion: there was no smear layer, and all dentin tubuli were open and clean, (e) 40% red betel leaf infusion: there was no smear layer, and all dentin tubuli were open and clean.

used to see the cleanliness of the canal wall, and can also be considered as one of the parameters of the successful results of the root canal preparation.<sup>23-27</sup>

Based on the assessment results conducted by using photomicrographs, it can be seen that in the control group irrigated with aquadest there was nonhomogeneous smear layer that covered the entire root canal wall. This is because aquadest has no active substances, such as saponins, and only serves to water the root canal walls but without any abilities to dissolve the smear layer, so it can not clean the root canal walls of the smear layer.<sup>28,29</sup> Meanwhile, in the two treatment groups irrigated with red betel leaf infusion with concentrations of 10% and 20%, there were homogeneous smear layers covering the roots, but there were few open dentin tubuli. This suggests that red betel leaf infusion with those concentrations contains little saponins, so it still has not worked effectively to clean the root canal walls of the smear layer.<sup>30</sup>

While the other two treatment groups irrigated with red betel leaf infusion with concentrations of 30% and 40% did not have smear layer so that all dentine tubuli could be open and clean. Both those groups even showed the same quality of cleaning power that was not statistically and significantly different. This is because at these concentrations the amount of active substance, saponin, contained in red betel leaf infusion at concentrations of 30% and 40% could work well and effectively to dissolve smear layer.<sup>21,31,32</sup>

Red betel leaf infusion actually contains active substance, saponin, a type of glycoside found in many plants with characteristics as "surfactants".<sup>4,7,12</sup> Saponins (phytonutrients) has properties resembling soap, so often called as "natural detergent", that is a foaming solution classified by the structure of the complex aglykon into triterpenoid and steroid saponins.<sup>4,26,33</sup> This compound has a long hydrocarbon chain with ion tip which consists of clusters of non polar (hydrophobic) and polar (hydrophilic) groups. Non-polar (hydrophobic) cluster will divide smear layer molecules into smaller particles so that water is not only easy to form an emulsion with smear layer, but also easy to be separated. Meanwhile, polar (hydrophilic) cluster will be dissolved in water to form bubbles and binding particles of smear layer causing the formation of emulsion.<sup>32-34</sup> However, because of the long hydrocarbon chains, not all smear layer molecules are soluble in water so that saponins will emulsify or suspense smear layer in water.

In this process, anions form colloidal micelle particles, the collections of (50-150) molecules, which hydrocarbon chain cluster with the ion tips facing the water so that the ions have higher solubility than the single insoluble ones.<sup>4,11,35</sup> The longer the hydrocarbon chain is, the higher value of solubility is. Thus, the ability of saponin to dissolve smear layer is getting better. Therefore, saponins can be characterized based on its ability as a "surfactant" that is capable of lowering the surface tension of root canal walls, so the smear layer containing dentine debris, organic materials, inorganic materials, and microorganisms in root canals can be dissolved later.<sup>36-39</sup> It can be concluded

that red betel leaf infusion with a concentration of 30% is effective for cleaning the root canal walls of the smear layer.

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