Research Report

Comparison of ratios combination calcium hydroxide $Ca(OH)_2$ with red pine (*Pinus densiflora*) of viscosity

Latief Mooduto,[1] Ari Subiyanto,[1] and Shafa Prasita[2]
[1]Staff of Conservative Dentistry Department, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia
[2]Student of Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Background: *Calcium hydroxide is a gold standard for root canal sealing material. However, there are many defects in calcium hydroxide that have prompted many researchers to look for alternative materials, including natural origin ingredients. Red pine (Pinus densiflora) is often used in dentistry for its antioxidant and antibacterial properties. The mixture of calcium hydroxide and red pine has never been achieved before. One of the criteria for material sealing is that the channel should be fully sealed with high flow ability and low viscosity. Therefore, this research is performed to analyze the viscosity value of the mixture of calcium hydroxide and red pine (Pinus densiflora).* ***Purpose:*** *Knowing the difference in viscosity of the combination of calcium hydroxide and red pine with a ratio of 1:1, 1:1.5, and 1:2.* ***Methods:*** *This study used four treatment groups, with each group consisting of 8 replications. Group 1 was a combination of calcium hydroxide and red pine with a 1: 1, group 2 the ratio was 1: 1.5, group 3 had the ratio 1: 2, and the positive control group used calcium hydroxide and a sterile aquadest. Calcium hydroxide in powder form and an extract of red pine in liquid form are mixed according to the ratio. The viscosity value is measured using a viscosity tester, namely the Brookfield Viscometer.* ***Results:*** *The control group had a lower viscosity than group 3, group 3, and group 2 than group 1.* ***Conclusion:*** *Combination of calcium hydroxide and red pine with a ratio of 1:2, the lowest viscosity was obtained compared to the ratio of 1: 1 and 1: 1.5.*

Keywords: *Calcium hydroxide; Red pine (Pinus densiflora); viscosity*

Correspondence: Ari Subiyanto, Conservative Dentistry Department, Faculty of Dental Medicine, Universitas Airlangga. Jl Mayjen. Prof. Dr. Moestopo No. 47, Surabaya 60132, Indonesia. Email : arisubiyanto57@gmail.com

INTRODUCTION

Root canal is a treatment that aims to repair an infected tooth [1]. Root canal treatment consists of three stages (*endodontic triad*), namely 1) preparation to form and clean root canals, 2) root canal sterilization to kill bacteria in the root canal, and 3) obturation or filling of the root canal [2]. As many as 58% of root canal treatment failures were caused by low quality obturation. The definition of obturation according to the *American Association of Endodontics*, is a method for filling and closing the root canals using obturation material [3]. Obturation material and the selection of obturation technique are important factors in the success of obturation [4].

Obturation material consists of a core material and a root canal sealer or paste. Root canal sealer or paste has the ability to create an attachment between the *gutta-percha* and the root canal wall which makes the root canal system completely closed thereby preventing root canal treatment failure from occurring [4]. Therefore, the sealer can affect the obturation results and thus affect the success of endodontic treatment [5]. The requirements for a good sealer must have a good adhesion between the core material and the root canal wall, be bacteriostatic, biocompatible to tissue, not irritate the periradicular tissue and not hinder tissue repair, on the contrary it helps repair injured tissue [3].

The sealer must also have a flowability that can penetrate the accessory canals in the root canal system. According to *American Dental Association* (ADA) specification No. 57 (*American National Standards*/ ADA 1983) and ISO-6876 (ISO 2001), the sealer must have a flowability of at least more than 20 mm of diameter. The higher the flowability, the higher the sealer's ability to penetrate irregular root canal systems [6].

The flowability of a liquid is closely related to its viscosity. Viscosity is defined as the degree of thickness of a liquid [7]. The relationship between viscosity and the flowability of a liquid is inversely proportional [8]. The low viscosity sealer has a watery texture, so the sealer has high flowability and the sealer is easy to penetrate in narrow and complex root canal systems, and vice versa. High flowability is required by the sealer because the sealer can completely cover the root canal so that the objective of obturation is fulfilled [6].

Calcium hydroxide is one of the sealers commonly used in endodontic treatments, because it has antibacterial properties, reduces tissue inflammation and can stimulate the formation of hard tissue because it can release calcium ions (Ca^{2} +) and hydroxyl ions (OH-). Calcium hydroxide as a root canal sealer also has disadvantages, which can cause leakage at the marginal edges because the sealer cannot cover the root canal which is narrow and complex as a whole [5,9]. The most recent study on the viscosity of calcium hydroxide sealers was carried out in 2015 under the brand *Sealapex*. The sealer has a high viscosity of 523,606 ± 20.73 Pa.s (Pascal: unit of viscosity) with a flowability of less than 20 mm, so it is not in accordance with the provisions of the flowability of ISO and ADA [6,10].

The viscosity of a liquid can be influenced by several factors including pressure, temperature, molecular size and weight, bonds between molecules, forces between molecules, the concentration of a substance, and the addition of other substances [11–14]. The viscosity increases with increasing molecular weight, calcium hydroxide has a molecular weight heavy enough that is 74.08 g / mol so that the viscosity owned too high calcium hydroxide sealer [15]. Due to some calcium hydroxide deficiency, it is combined with herbal ingredients that can increase anti-bacterial power, and reduce its viscosity by having a watery texture, so that the flowability of the sealer is high and the sealer is easy to penetrate in narrow and complex root canal systems. Evaluation of simple physical properties such as viscosity needs to be carried out on the sealer because it can affect its flowability [16].

Herbal ingredients or natural products are becoming more popular nowadays due to their high antimicrobial activity, biocompatibility, anti-inflammatory and anti-oxidant properties [17]. One of these natural ingredients is *red pine*. *Red pine* or *Pinus densilora* is a red pine plant that grows naturally or is planted in mountainous areas of Korea, Japan, and China [18]. The leaves have long been used in traditional Korean medicine for liver problems, indigestion, and skin diseases [19]. In its biological properties, *red pine* has antibacterial properties because it contains antibacterial ingredients such as flavonoids [20,21]. In its physical properties, *red pine essential oil* has a viscosity of 12 Pa.s which is low. This viscosity is obtained by *red pinered pine* because *essential oil* contains 23.6% water, so *red pine* has a low viscosity [22].

In 2019, a study was conducted on the comparison of the antibacterial power between *red pine (Pinus densilora)* and *green pine (Pinus merkusii)* which is native to Indonesia. From the research results, the concentration of *red pine* which is lower than the concentration of *green pine*, it has been able to provide antibacterial activity [23,24]. In the same year, there was also a study that discussed the comparison of the antioxidant power between *red pine (Pinus densilora)* and *green pine (Pinus merkusii).* From this research, it was found that the antioxidant activity in *red pine (Pinus densilora)* was higher than that of *green pine (Pinus merkusii)* [25]. Based on some existing research related to the use of extract *red pine (Pinus densilora),* until now there has been no research regarding its use as an alternative sealer material.

Therefore, research is needed on the use of *red pine (Pinus densilora)* combined with calcium hydroxide powder as an alternative sealer material for its physical properties, namely viscosity. This research was conducted at the Industrial Research and Consultation Center (BPKI) Surabaya, using extract *red pine* with a concentration of 0.78% which is the minimum killing concentration (KBM) and calcium hydroxide using a ratio of *powder / liquid* 1: 1, 1: 1.5 and 1. : 2 [24]. Ratio *Powder / liquid* 1: 1; 1: 1.5 and 1: 2 were chosen because, the ratio of 1: 1 is the *gold standard* recommended by the manufacturer, the ratio of 1: 1.5 and 1: 2 was chosen because there has been no previous research regarding the combination of calcium hydroxide and *red pine* so that it follows the ratio of calcium hydroxide use as a sealer combined with propolis.

MATERIALS AND METHODS

This research is a laboratory experimental research with a *posttest only control group design*. In this study, there were 4 treatments to be given and based on calculations with Federer's formula, the minimum sample size required for each sample group was 6. In this study each group of experimental units consisted of 8 replications.

The research sample was divided into 4 groups to determine the difference in viscosity of the combination of calcium hydroxide and *red pine (Pinus densilora)* with a ratio of 1: 1, 1: 1.5 and 1: 2, consisting of group I, namely a combination of calcium hydroxide with extract *red pine(Pinus densilora) with a* ratio of 1: 1, group II, namely a combination of calcium hydroxide with extract *red pine (Pinus densilora)* a 1: 1.5 ratio, group III, a combination of calcium hydroxide with extract *red pine (Pinus densilora)* a 1: 2 ratio, and group IV is a combination of calcium hydroxide and sterile distilled water as a positive control with a ratio of 1: 1.

In this study, the researchers obtained the *essential oil* of *red pine (pine densilora)* leaves in capsules from Seoul National University, Korea. *essential oil* leaf *Red pine (pine densilora)* with a content of 100% is taken as much as 0.78 ml. Then dilution was carried out by adding 100 ml sterile distilled water and 3 drops of glycerin for the emulsion process so that the mixture was homogeneous until it reached a concentration of 0.78%.

The combination of calcium hydroxide and *red pine (Pinus densilora) is* made by mixing calcium hydroxide powder and *red pine (Pinus densilora)* with a ratio of 1: 1, namely mixing 10 grams of calcium hydroxide powder with extract *red pine (Pinus densilora)* 10 ml, ratio 1: 1.5 mixing 10 grams of calcium hydroxide powder with extract *red pine (Pinus densilora)* 15 ml, while the ratio of 1: 2 is mixing 10 grams of calcium hydroxide powder with extract *red pine (Pinus densilora)* 20 ml and as a control group, calcium hydroxide is mixed with sterile distilled water with

1: 1 ratio, namely mixing 10 grams of calcium hydroxide powder with 10 ml of sterile distilled water.

Calcium hydroxide is added with *red pine (pine densilora)* 0.78% according to the ratio of each group, then stirred for 10 minutes until homogeneous and the consistency becomes a paste emulsion with units of grams / ml. Viscosity testing uses the Brookfield viscometer, by setting up and turning on the Brookfield viscometer so that the viscometer is ready for use, then selecting *spindle* the appropriate, then installing the *spindle* on the viscometer and changing the number *spindle* listed on the viscometer according to the number *spindle* selected, then entering the sample or paste to be tested into the measuring cup and put it into the Brookfield viscometer so that the *spindle* touches the paste in the measuring cup, followed by pressing the motor button so that the spindle can rotate 100 RPM (revolutions per minute) and ensure that the percentage value on the viscometer is in the range : 10-100%. If $<10\%$ increase the spinning speed and use a larger spindle. If $>100\%$ reduces the spinning speed and uses a smaller spindle and the latter reads and records the results shown by the tool.

RESULTS

Table 1 shows that the highest viscosity value belonged to group I with a value of 25.7350 Pa, higher than group II with a value of 25.3125 Pa. Meanwhile, the lowest viscosity belongs to group III with a value of 24.1125 Pa. The results of the Kolmogorov-Smirnov test that the data in each study group has a significance value greater than 0.05. This shows that the data in each group are normally distributed. The results of the Levene test which aims to determine the homogeneity of the data and it can be seen that the significance value is greater than 0.05 ($p>0.05$) which is equal to 0.354 which indicates that the data variation is homogeneous. The results of the One Way Anova test, F counts 132.819 with sig. 0.000. Thus, it can be concluded that H0 is rejected, or that there is a significant difference between the treatment groups. The results of the multiple comparisons difference test using Tukey HSD with the results of a significant difference between one study group and another. This means that the combination of calcium hydroxide and red pine with a ratio of 1: 1, 1: 1.5, and 1: 2 has a significant difference in viscosity.

Table 1. Mean value and standard deviation of viscosity combination of calcium hydroxide - red pine

Ex.	Combination	Mean Viscosity (Pa)
I	$Ca(OH)_2$: *Red Pine* 1 : 1	25.7350
II	$Ca(OH)_2$: *Red Pine* 1 : 1.5	25.3125
III	$Ca(OH)_2$: *Red Pine* 1 : 2	24.1125
IV	$Ca(OH)_2$: *Sterile Aquadest* 1 : 1	22.1250

DISCUSSION

The results showed that the highest viscosity was in group I at 25.7350 Pa.s followed by group II at 25.3125 Pa.s and the lowest in group III at 24.1125 Pa.s. The ISO and ADA specifications do not list viscosity measurements for root canal sealers. The viscosity of the combination of calcium hydroxide and *red pine* decreased inversely with the increasing ratio of extract *red pine* to calcium hydroxide. This shows that the addition of extrac*t edible pine (Pinus densilora)* has an effect on decreasing viscosity.

The results showed that the combination of calcium hydroxide and *red pine (Pinus densilora)* with a ratio of 1: 1, 1: 1.5 and 1: 2 had a significant difference in viscosity. The increase in the ratio of extract *red pine* to calcium hydroxide caused the viscosity to decrease significantly according to the results of the study. This is because by increasing the extract *red pine* in the combination of calcium hydroxide and *red pine*, the structure of the combination will have an effect and result in decreased viscosity. There are several things that can affect the viscosity of the combination of calcium hydroxide with *red pine (Pinus densilora).*

The viscosity of a liquid can be influenced by several factors including the bonds between molecules, intermolecular forces, molecular size and weight, pressure, temperature, the concentration of a material, and the addition of other substances [11–14] Interactions between molecules can affect viscosity, the greater the interaction between molecules a liquid then the liquid has a high viscosity or viscosity because of the high attraction between the molecules in the liquid [12]. The interaction between molecules can be obtained from chemical bonds and forces between molecules which are divided into three, namely the disperse force, the dipole-dipole force and the hydrogen bonds present in a compound [26]. The interactions between molecules can be determined from the strength of the chemical bonds and the forces between the molecules in the combined sealer of calcium hydroxide and *red pine*. This inter-molecular interaction is based on the presence of cohesive force or attraction between the same or different molecules in a compound, the stronger the interactions between molecules, the more viscosity will be increased [45].

In the combination sealer of calcium hydroxide and *red pine,* there are hydrogen bonds that come from the reaction between aromatic compounds, *red pine* namely flavonoids and phenolic acids with water. These aromatic compounds are included in the polyphenol group which are hydrophilic compounds, namely compounds that are very easy to bond with water because they contain OH bonds or hydroxyl groups which will then react with water to form hydrogen bonds. Hydrogen bonds are formed between the hydrogen atom (H) and the highly electronegative elements, namely, nitrogen (N), oxygen (O) and fluorine (F). Hydrogen bonds are weak when compared to ionic and covalent bonds [26,27]. The more hydrogen bonds there will be an impact on the weaker interactions between molecules so that the viscosity decreases. This is consistent with research, the more extract is added *red pine*, the more aromatic compounds that react with water form hydrogen bonds. The more hydrogen bonds that are formed, the weaker the interactions between molecules will be, so that each addition of the extract *red pine* viscosity decreases significantly.

The combination sealer of calcium hydroxide and *red pine* also produces an intermolecular force, namely the dipole-dipole force which is the van der waals force. The van der waals force is a force and a bond between molecules which is weak [28,29]. The van der waals force on this sealer is formed from polar molecules that are bonding to each other. The hydroxyl ion in calcium hydro cyclone is a polar compound and the aromatic *red pine* compound is also a polar compound because it comes from the polyphenol group which also contains hydroxyl ions. Hydroxyl ion is hydrophilic, that is, it dissolves easily in water so the compound is polar. Polar molecules that interact with each other will produce van der waals forces which are weak bonds compared to ionic, covalent or hydrogen bonds [26]. The presence of a large number of hydrogen bonds accompanied by a weak van der waals force will weaken the interaction between calcium hydroxide molecules and aromatic compounds *red pine*. Weak intermolecular bonds will decrease viscosity, because weak intermolecular attractive forces affect the familiarity of a liquid [12]. This is in accordance with the research, the more extract is added *red pine*, the more aromatic compound hydroxyl ions will interact with calcium hydroxide hydroxyl ions to form a lot of van der waals forces with weak properties. The more van der waals forces with weak properties are formed, the weaker the interaction between molecules will be, so that each addition of extract *red pine* viscosity decreases significantly.

In the combination sealer of calcium hydroxide and *red pine,* there will be an acid-base reaction between calcium hydroxide and aromatic compounds *red pine.* This acid-base reaction will affect the viscosity. The acid-base reaction is one of the neutralization chemical reactions which in the process will produce a product in the form of water. Calcium hydroxide is a strong base compound that reacts easily with acids, while extract *red pine* contains aromatic compounds, one of which is phenolic acid, which is a weak acid derived from polyphenols [22,30–32]. The reaction between phenolic acid and calcium hydroxide will produce a phenolic calcium salt and a byproduct, namely water [21]. The water product from this reaction will decrease the viscosity due to the decreasing degree of viscosity. This is in accordance with research, the more extract is added *red pine*, the more phenolic acids will react with calcium hydroxide to form an acid-base reaction. If the water production in the combination of calcium hydroxide and extract *red pine* increases because of the increased acid-base reaction, it will decrease the viscosity of a liquid, so that each addition of extract the *red pine* viscosity decreases significantly.

The size and size of the particles can affect the viscosity of the sealer combined with calcium hydroxide and *red pine*. Calcium hydroxide has a heavy molecular weight of 74.08 g / mol, when the ratio of calcium hydroxide powder decreases, the size and particle weight of the combined sealer of calcium hydroxide and *red pine* also decreases. Decreasing the size and molecular weight will decrease the viscosity of a material [12]. Extract *Red pine* contains water, when the ratio of liquid *red pine* increases, the water content in the sealer combination of calcium hydroxide and *red pine* also increases. If the water production on a combination of calcium hydroxide and *red pine* to grow it will reduce the viscosity of a fluid [33–36]. This is in accordance with the research, the more the addition of extract *red pine*, the lower the ratio of calcium hydroxide so that the size and weight of the calcium hydroxide and sealer particles *red pine* also decreased. The more the extract ratio, the *red pine* more water there is, so that each addition of extract *red pine* viscosity decreases significantly.

Pressure is directly related to viscosity, pressure on a liquid will have an effect on increasing cohesion force, so that the higher the pressure the higher the viscosity, but in this study the sealer combination of calcium hydroxide and *red pine is* not given pressure so in this study the pressure cannot affect the decrease. significant viscosity [12].

The relationship between temperature and viscosity is inversely related, heating of a liquid causes the existing molecules to gain energy to move so that the interaction force between molecules weakens, thus the viscosity of a liquid will decrease with an increase in temperature [12]. The temperature used in this study was 37^0 Celsius which is room temperature, but treat the temperature of each group the same because temperature is a control variable, so in this study the temperature could not affect the significant decrease in viscosity.

Viscosity is directly proportional to the concentration of a liquid, if a liquid has a high solution concentration, then the viscosity of the liquid will be high [13]. The concentration of extract *red pine* used in this study was a concentration of 0.78% which is the minimum killing concentration (KBM), in this study the concentration used in each treatment group was the same, so in this study the liquid concentration also could not affect the decrease in viscosity. significant.

In this study, another substance was added, namely glycerin. Glycerin can increase viscosity [11,14]. In this study, glycerin helps in diluting extract *red pine. Red pine* is a substance that is obtained from the extraction of leaves *pine densilora* in the form of essential oil capsules with 100% levels. The dilution of *red pine was* carried out with sterile distilled water and a little glycerin until it reached a concentration of 0.78%, which is the minimum killing concentration (KBM). Glycerin functions for the emulsion process, namely the union between sterile distilled water and oil *red pine* so that the mixing is homogeneous [37]. In this study, the use of glycerin was the same for each treatment group, so in this study the addition of glycerin also did not affect a significant decrease in viscosity.

The viscosity obtained by positive control was lower than that of the combination group of calcium hydroxide and *red pine.* This could be due to the fact that the positive control carried out a combination of calcium hydroxide and sterile distilled water without the addition of extract *red* *pine*. The addition of extract *red pine* can increase viscosity when compared to without the addition of extract *red pine*. Extract *red pine* contains 0.78% essential oil. Oil or oil has a higher viscosity than water, because oil has a molecular weight greater than the molecular weight of water [12,38,39]. The lower the molecular weight of a compound the lower its viscosity. So that the combination sealer of calcium hydroxide and distilled water is sterile or positive control has a lower viscosity than the combination sealer of calcium hydroxide and *red pine*.

Extract *Red pine* contains gallic acid or gallic acid which is a compound group of phenolic acids. Gallic acid which is owned by *red pine* contains pyrogallol groups which can bind to the dentine surface of root canals [40–42]. The bond that occurs between gallic acid *red pine* and dentin makes the combined sealer of calcium hydroxide and *red pine* have an adhesion force to dentin. In this case the adhesion force will allow the sealer to prevent leakage at the marginal edge [43,44]. The adhesion force also affects the ability of the sealer to flow to the dentin to be higher because of the attractive force between the particles of a liquid and the particles on the dentin.

The sealer combination of calcium hydroxide and *red pine* can also inhibit bacterial growth and stimulate the formation of new tissue. This is obtained because calcium hydroxide has a mechanism of action to release calcium ions (Ca2 +) and hydroxyl ions (OH) [5]. Calcium hydroxide must be combined with liquid because calcium hydroxide powder is difficult to enter into the root canal and fluid is also needed to release the hydroxyl ion, in this case the liquid used is extract *red pine (Pinus densilora).* The release of calcium ions (Ca2 +) can play a role in the process of tissue mineralization, while the release of hydroxyl ions (OH-) can increase the pH of the calcium hydroxide sealer. The increase in pH can form an alkaline environment causing damage to the cytoplasmic membrane of the bacteria resulting in a protein denaturation process which will inhibit the DNA replica of bacteria and cause inhibition of bacterial growth. In addition, the anti-bacterial effect is also obtained from essential oil *red pine* which contains several antibacterial ingredients including phenolic acid, flavonoids, gallic acid, terpenoids, tannins, alkaloids, α-pinene, β-pinene, and limonene [22,30].

Therefore, the combination sealer of calcium hydroxide and *red pine* can be used as a candidate as a root canal sealer in endodontic treatment because the research results show that the sealer has a low viscosity and has a watery texture, so that the sealer has high flowability and the sealer is easy to penetrate the duct system. narrow and complex roots. High flowability is required by the sealer because the sealer can completely cover the root canal so that the purpose of obturation is fulfilled. Adhesion force of the sealer to the dentin is also needed to prevent leakage at the marginal edges because the sealer can cover the whole narrow and complex root canal. In addition to the ability to flow and the existing adhesion force, the mechanism of action of these sealers can also inhibit bacterial growth and stimulate the formation of new tissue.

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