

Research Report

Effectiveness of Sealer Combination Zinc Oxide (ZnO) with Red Pine (*Pinus densiflora*) on Viscosity and Solubility (Laboratory Experimental)

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ABSTRACT

Background: Failure of endodontic treatment is caused by 60% of poor obturation. Sealers have an important role in the obturation process, that is filling the root canals and the space between the dentin and the core material. Sealers must have low viscosity so that they can flow easily and fill the entire root canal space so can form a good seal and have physical properties that are not easily soluble to oral fluids to prevent sealer degradation which can cause gaps during filling and become a medium for bacterial proliferation. Red pine extract is a natural ingredient that contain flavonoid and phenolic acid that are effective in reducing the viscosity and solubility of the sealer combination ZnO with red pine.

Purpose: To determine the effectiveness of sealer combination ZnO with red pine in reducing viscosity and solubility.

Methods: Red pine (*Pinus densiflora*) extract with a concentration of 100% was diluted to a concentration of 0.78% using the dilution formula $M1.V1 = M2.V2$. Viscosity test was done by mixing ZnO with red pine extract 0.78% 1: 1 and calcium hydroxide ($Ca(OH)_2$) with 1: 1 sterile aquadest as a comparison, then each sample was measured for viscosity using a brookfield viscometer. The solubility test was carried out by placing the paste on a disc with a diameter of 15 mm x 3 mm and then being set and immersed in distilled water for 24 hours then the percentage of solubility was calculated.

Results: The viscosity value of combination ZnO with red pine is 19.89 Pa.s and the solubility is 0.0075%, while $Ca(OH)_2$ with sterile aquadest had a viscosity of 23.32 Pa.s and a solubility is 0.029%. **Conclusion:** The combination of ZnO with red pine is effective in reducing the viscosity and solubility of the combination sealer. The viscosity and solubility of the combined sealer is lower than $Ca(OH)_2$ with sterile aquadest.

Keywords: Combination of ZnO – Red pine; Calcium hydroxide; Red pine (*Pinus densiflora*); Viscosity; Solubility

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INTRODUCTION

Root canal treatment is a treatment of dental conservation that aims to maintain damaged teeth to be biologically accepted by the surrounding tissue without any signs of pathological abnormalities and still functional in the oral cavity.^{1,2} Root canal treatment failure in 60% is due to poor obturation (root canal filling). The filling of the root canals is the final stage that requires attention since the anatomy of the root canals and the anastomoses of the canals always differ, making it challenging to perform the obturation process.²

Sealers have an essential role in the obturation process by filling the space between the dentin and the core material.³ There is still no ideal sealer. According to Grossman (2014),⁴ ideal sealer requirements are easy to inject into the root canal, fills the lateral and apical canals, does not shrink after injection, moisture resistant, radiopaque, does not color teeth, does not irritate periapical tissues, if required,

easy to extract from the root canals, bacteriostatic, sterile and long-setting time to get enough working time

Viscosity is a measure of the fluid's viscosity and an important indicator to determine the flow characteristics of the sealer.⁵ Due to its lower resistance of the liquid to flow (high flow), good flowability of the sealer is accomplished with low viscosity such that the sealer can flow freely and fill the entire space in the root canal.^{5,6} The ISO and ADA specifications regarding the sealer's flow do not include a viscosity measurement but instead use a measurement of the sealer film's diameter between two glass plates (ANSI / ADA 2000, BS EN ISO 6876 2002), which relates to viscosity but is easier to measure. According to ISO 6876/2001 and ADA No. 57, the root canal sealer flow is > 20 mm.^{7,8}

Sealers must be resistant to oral fluids or not dissolve easily. According to the International Standard and ANSI / ADA specification No. 57 and No. 30, the sealer solubility should not exceed 3% by mass fraction after immersion in

water 24 hours. Degradation of the sealer can cause gaps and voids on the dentin's surface with a sealer or gutta-percha with a sealer at the time of filling and become a medium for bacterial proliferation colonization.⁹¹⁰

Zinc Oxide Eugenol (ZOE) is one of the sealers used in root canal treatment because it has an antimicrobial effect, mild analgesic, adequate radiopacity, economical price, easy to obtain and apply. ZOE consists of a powder in the form of zinc oxide (ZnO) and a liquid in the form of eugenol. The eugenol contained in the zinc oxide sealer eugenol has the disadvantage that cytotoxic can irritate the periapical tissue, resulting in necrosis of the bones and tooth cementum.¹¹¹²

ZOE sealer has a high viscosity, namely Grossman's 3: 1 of 105,90 Pa at 25°C. *Tubliseal EWT* zinc oxide eugenol sealer has a viscosity that is 61,95 Pa at 25°C, and viscosity is 27,34 Pa at 37°C.¹³¹⁴¹⁵ Meanwhile, calcium hydroxide-based sealers, widely used as gold standards, have a viscosity of 26.14 Pa for Apexit.¹³¹⁶ ZOE sealers have high solubility compared to other sealer materials; there are 0.73% and 0.77%, so they are more susceptible to microleakage of $2,426 \pm 0.733$ solubility level of ZOE sealers is still within ISO and ADA standards.⁹¹⁷

Herbal ingredients are biocompatible, anti-bacterial, analgesic, anti-inflammatory, and antioxidant; different dental materials are currently being manufactured from herbal ingredients based on these properties.¹⁸¹⁹ Red pine (*Pinus densiflora*) is a type of pine that grows naturally in the mountainous regions of Korea, China, and Japan, has many pharmacological properties, such as anti-inflammatory, anti-aging, anti-bacterial, and antioxidant effects. One of the essential ingredients of *Pinus densiflora* leaf oil is flavonoids and phenolic acids.²⁰²¹²² Red pine essential oil has a viscosity of 12 cSt, which is low; the combination of zinc oxide (ZnO) and red pine essential oil is an acid-base reaction that will produce water products.²²²³²⁴

Based on this background, the authors want to study the efficacy of the zinc oxide and red pine combination sealer to minimize viscosity and solubility, which is then compared to calcium hydroxide, the gold standard root canal sealer.

MATERIALS AND METHODS

This research is experimental laboratory research, using two kinds of samples, namely the sample in the form of a paste emulsion used for viscosity testing and the sample set and placed in the mold for solubility testing. The tools and materials used were red pine extract with a concentration of 0.78%, zinc oxide powder, calcium hydroxide (Ca(OH)₂) powder pro analysis, sterile aquadest, Brookfield viscometer, digital scales, cement spatula, and samples sized 15 mm x 3 mm, glass slab, beaker glass, nylon thread.

In this study, essential oil for red pine (*Pinus densiflora*) leaves with a concentration of 100% was diluted to 0.78% using the formula for the ratio of concentration and volume:

$$M1.V1 = M2.V2$$

Note: M1: Initial concentration (%);

V1: Initial volume (ml);

M2: Final concentration (%);

V2: Final volume (ml)

A total of 0.78 ml of red pine extract was added with sterile aquadest as much as 99.22 ml and then added 3 drops of glycerin for the emulsion so that the mixture was homogeneous until it reached a concentration of 0.78%. There were two treatment groups, namely treatment group I (a combination of zinc oxide with red pine) and treatment group II (a combination of calcium hydroxide and sterile aquadest as a comparison). Each treatment group consisted of 18 replications.

The combination of zinc oxide and red pine (*Pinus densiflora*) was made by mixing zinc oxide and red pine powder with a 1: 1 ratio, namely mixing 10 grams of zinc oxide powder with 10 ml of red pine extract, and as a comparison group, namely Ca(OH)₂ powder pro analysis. Mixed with sterile aquadest using a 1: 1 ratio, namely mixing 10 grams of Ca(OH)₂ powder with 10 ml of sterile aquadest. The two treatment group samples were then stirred for 10 minutes until they were homogeneous, and the consistency became a paste emulsion.

A cleaned and prepared Brookfield viscometer was used to perform a viscosity test of the sealer combination of zinc oxide and red pine and calcium hydroxide and sterile aquadest. After that, for measurements using a Brookfield viscometer, placed the paste into a beaker glass by first turning on the viscometer, then choosing the spindle to be used. The rotation of the spindle in the paste indicates the importance of the paste's viscosity. According to the selected spindle number, install the spindle on the viscometer after that and adjust the spindle number printed on the viscometer. Next, put the beaker containing the sample on the viscometer and press the "motor" button so that the spindle can rotate, ensuring that the percentage value on the viscometer is in the 10-100% range. If the percentage value less than 10%, then increase the spinning speed and use a larger spindle and if it is bigger than 100%, reduce the spinning speed and use a smaller spindle.

The solubility test was performed by putting the paste on a mold/disc with a diameter of 15 mm and a height of 3 mm. Then put 100 percent moisture in an incubator at 37°C until set. Every disc was tied with nylon thread, and analytical balances were used to calculate the initial mass (W₀). The samples were then immersed in 20 ml of deionized water and incubated for 24 hours in 100% humidity at a constant temperature of 37°C.²⁵

Solubility evaluation (%): After immersing in water for 24 hours, the disc is removed from the tube, dried with blotted paper, left for 24 hours to dry completely, and then weighed again. The following equation calculates the solubility amount (%):²⁵

$$\text{Solubility (\%)} = \frac{W_0 - W_f}{W_0} \times 100$$

Note:

W₀ = Initial weight

W_f = Final weight after immersion

Data processing used statistical analysis test with the degree of significance or p value= 0.05 or 95%: *Kolmogorov-Smirnov Test* to determine data distribution (normality); Homogenous test using *Levene test*; *One-way ANOVA* to determine the significance of all treatment groups; *Mann Whitney* to test the differences between study groups, if the data were not normally distributed and or were not homogenous.

RESULTS

This study consisted of two units of the research group, namely treatment group I (the combination of zinc oxide (ZnO) - red pine (*Pinus densiflora*) with a ratio of 1: 1), while treatment group II was the comparison (the combination of calcium hydroxide-sterile aquadest with a comparison 1: 1), with each unit the research group consisted of 18 replications. The mean of the research results is shown in Table 1 and 2.

From the measurement of each study group's viscosity and solubility values, the lowest viscosity value was owned by the ZnO + Red Pine group with a value of 19.89 Pa.s. As for the solubility, the results showed that the lowest solubility value was owned by the ZnO + Red Pine group with a value of 0.0075%.

Table 1. The mean viscosity value of Ca(OH)₂ + Aquadest sterile and ZnO + Red Pine with a powder/liquid ratio of 1: 1 (Pa.s)

	Ca(OH) ₂ + Aquadest steril (1 : 1)	ZnO + Red Pine(1 : 1)
Amount of sample	18	18
Mean	23.3188889 ≈ 23.32	19.8922222 ≈ 19.89

Table 2. The mean solubility value of Ca(OH)₂ + Sterile aquadest and ZnO + Red Pine with a powder/liquid ratio of 1:1 (%)

	Ca(OH) ₂ + Aquadest steril (1 : 1)	ZnO + Red Pine(1 : 1)
Amount of sample	18	18
Mean	0.02877778 ≈ 0.029	0.0075

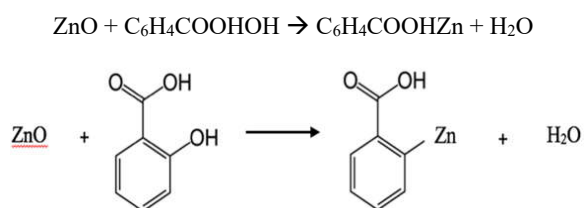


Figure 1. The reaction of zinc oxide with salicylic acid produces zinc salicylate and water.

DISCUSSION

This study found that the combination sealer of zinc oxide and red pine had a lower viscosity and solubility compared to calcium hydroxide and sterile aquadest. In this study, red pine extract with a concentration of 0.78% was used because it has an anti-bacterial effect against *Enterococcus faecalis*, which often causes root canal treatment failure.²⁶ For the powder/liquid (P/L) comparison, previously the researchers had conducted a preliminary test, namely the viscosity test of the combination of zinc oxide and red pine using three P/L ratios, namely 1: 1, 1: 2, 1: 3, because until now There have been no studies using zinc oxide powder with liquid red pine. From the three comparisons, the results show that 1: 1 has the closest viscosity to the combination of calcium hydroxide and sterile aquadest, which is the gold standard root canal sealer.

The viscosity of the sealer combination of zinc oxide and red pine is low due to the weak interactions between molecules in the form of hydrogen bonds and Van Der Waals forces (dipole-dipole forces), which are weak types of bonds and forces between molecules as well as acid-base chemical reactions that produce water products.^{27,28}

The interaction between molecules is based on the cohesive strength and attraction between the same or different molecules in a compound; the stronger the interaction between molecules, the greater the substance's attractive characteristic, such that the substance's viscosity or viscosity level increases, and vice versa if the interaction between weak molecules is due to chemical bonds and weak molecules increases²⁹.

The hydrogen bonding in the zinc oxide sealer with red pine comes from the aromatic red pine compound, namely flavonoids and phenolic acids, a group of polyphenols containing hydroxyl ions (OH⁻), which easily bind to water and then form hydrogen bonds. Hydrogen bonds are weak when compared to ionic and covalent bonds.²⁸

Van Der Waals' dipole-dipole forces are weaker than those of the ion-dipole forces produced by the calcium hydroxide combination with the sterile aquadest. Zinc oxide, a polar compound that binds to the aromatic red pine compound, a polar compound because it contains hydroxyl ions, is the Van Der Waals power (OH⁻)²⁸.

Acid-base reactions occur between aromatic red pine phenolic acids, which are weak acids of the polyphenol group, one form of phenolic acid, namely salicylic acid.³⁰ Amphoteric zinc oxide is a compound that can act as an acid or a base, depending on environmental conditions. In this case, ZnO acts as a base reacting with salicylic acid to produce zinc salicylate and water (Figure 1). This water production will decrease the combined sealer viscosity.^{24,28}

The viscosity of calcium hydroxide is higher than the sealer's viscosity combined with zinc oxide and red pine because the strong interactions between molecules come from ionic bonds, ionic bonds are bonds that occur between metallic (Ca²⁺) and non-metal (OH⁻) elements. In this ionic bond, there is a transfer between electrons, and it is a strong

chemical bond, stronger than the hydrogen bond and the Van Der Waals force found in the combination sealer of zinc oxide and red pine.²⁸

Also, there are fewer hydrogen bonds in calcium hydroxide than zinc oxide and red pine combination sealers, containing many polyphenol compounds.²⁷²⁸²⁹³⁰³¹³² Combination Ca(OH)₂ and sterile aquadest produce a dipole ion force; this force is stronger than the dipole-dipole force formed in the sealer combination of zinc oxide and red pine.²⁷³¹

The combination sealer of zinc oxide and red pine has a low solubility compared to calcium hydroxide and sterile aquadest. The weak interaction between molecules comes from the number of hydrogen bonds and Van Der Waals forces (dipole-dipole forces) and the combination sealer's low compound polarity. due to a benzene group, a nonpolar molecule, and a CH bond, which will prevent the absorption or entry of water into the sealer of the combination of zinc oxide and red pine.²⁷²⁸²⁹³¹³²³³ The stronger the interactions between molecules, the greater the tendency for the substance to dissolve in the solvent so that it has high solubility, and vice versa if the interactions between molecules are weak, the solubility of a substance is low.²⁹

The solubility of calcium hydroxide with sterile aquadest is higher than the combination sealer of zinc oxide and red pine due to strong interactions between molecules originating from ionic bonds, at least hydrogen bonds weak, and the presence of dipole ion forces. Also, the solubility of Ca(OH)₂ and sterile aquadest is high due to the dissociation reaction, the high polarity of the compound, and its hygroscopic properties.²⁷²⁸²⁹³¹³² Calcium hydroxide is a strong base which, when dissolved in water, a complete dissociation reaction will occur, becoming the constituent ions, namely Ca²⁺ and OH⁻ ions.²⁸ The hydrogen bonding in the hydroxyl ion will affect a substance's polarity because it is hydrophilic and binds very easily to water.³¹³² The hygroscopic characteristics cause calcium hydroxide to easily absorb water, which will encourage further reactions of powder and liquid and free additional Ca²⁺ and OH⁻ ions so that the solubility is high.³³

The viscosity value of the combination sealer zinc oxide with red pine is lower than that of the calcium hydroxide-based sealer on the market, namely Apexit with a viscosity of 26.14 Pa so that the combination sealer of zinc oxide and red pine can provide better flow.¹³¹⁶ In addition, the solubility of the sealer combination of zinc oxide and red pine of 0.0075% has met the ANSI / ADA standard, which is not more than 3% of the mass of the fraction after immersing in water for 24 hours.

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