



The Antibacterial Effectivity of Ethanol Extract of Red Grape (*Vitis vinifera* Variant Red globe) as a Root Canal Irrigation Material Against the Growth of *Actinomyces spp.* Bacteria in Vitro

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Abstract. *Actinomyces spp.* is a bacterium that can cause root canal failure. Many antibacterial materials have been used to kill *Actinomyces spp.*, especially natural materials for developing dentistry green economics. One is grapes (*Vitis vinifera variant Red globe*) because they have active antibacterial compounds, flavonoids, phenolic acids, and tannins. This study aims to determine the antibacterial effectivity of ethanol extract of red grapes (*Vitis vinifera variant Red globe*) as a root canal irrigation material against the growth of *Actinomyces spp.* bacteria in vitro. This study used a true experimental research using the dilution methods with 8 sample groups, consisting of K(-), K(+), 25%, 22,5%, 20%, 17,5%, 15%, dan 12,5%. Kruskal Wallis was statistical analysis tested to determine the significance of differences in the treatment group. Minimum Bactericidal Concentration (MBC) at a concentration of 15%. Statistical tests showed significant differences in the effect of ethanol extract of red grapes (*Vitis vinifera variant Red globe*) at each concentration in inhibiting the growth of *Actinomyces spp.* bacteria. In this study, the ethanolic extract of red grapes (*Vitis vinifera variant Red globe*) has antibacterial effectivity as a root canal irrigation material against the growth of *Actinomyces spp.* bacteria in vitro.

Keywords: Ethanol extract of red grapes · *Actinomyces spp.* · Minimum Bactericidal Concentration

1 Introduction

Root canal treatment is one of the pulp disease treatments in which the vital or necrotic pulp from the root canal is completely removed and replaced with root canal filling material. Root canal treatment aims to maintain the teeth to work correctly and last as long as possible in the oral cavity with three main stages that help the success of the treatment, preparation, cleaning, and obturation. Root canal preparation is done mechanically with a tool and chemically combined with root irrigation [1].

Root canal irrigation is carried out periodically using irrigating materials to remove necrotic tissue, microorganisms, and dentin powder. An ideal irrigant has a broad spectrum antibacterial effect, is non-toxic, can dissolve the remaining necrotic pulp tissue,

and prevents the formation of a smear layer. However, from various studies, no irrigation solution compound can meet these ideal criteria, including Sodium Hypochlorite (NaOCl) root canal irrigation agent [2, 3].

Root canal irrigant NaOCl is dentistry's most commonly used root canal irrigant. However, NaOCl has a weakness, one of which is not being able to eliminate resistant bacteria such as *Actinomyces* spp. This bacterium is one of the gram-positive facultative anaerobic bacteria commonly found in root canal infections, and it causes root canal failure. Therefore, alternative materials are needed that can be used as root canal irrigation materials [4].

The idea of a green economy received global enthusiasm, in 2009 the Organization for Economic Co-operation and Development (OECD). In Indonesia itself, green economy adaptation began with the 2017 UN Convention on Climate Change (UNFCCC). Currently, in dentistry for green economics, several natural materials have been developed as alternative materials for root canal irrigation because they have high antibacterial activity and low toxicity, one of which is red grapes. Grapes contain various nutrients, such as vitamins, minerals, carbohydrates, fiber, and phenolic compounds that have antibacterial activity. Research shows that grapes' skin, seeds, and flesh contain phenolic compounds with antibacterial potential [5].

See the antibacterial potential in grapes; it is necessary to research the antibacterial effectiveness of red grapes (*Vitis vinifera* variant *Red globe*) as an alternative root canal irrigation material against the growth of *Actinomyces* spp bacteria that can cause root canal treatment failure to support dentistry green economy.

2 Materials and Method

- 2.1. *Preparation of Red Grape Ethanol Extract.* The ethanol extract of red grapes was made using the maceration method with 96% ethanol solvent obtained from UPT Laboratory of Herbal Materia Medica Batu. 2 kg of red grapefruits were washed, dried, and ground using a blender. Red grape juice was soaked with 96% ethanol solvent for 24 h and then filtered using filter paper. The filtrate was evaporated with a Vacuum Rotary Evaporator to get 100% red grape extract. After that, the extract was diluted to make a concentration of 25%, 22.5%, 20%, 17.5%, 15% and 12.5%.
- 2.2. *Preparation of Bacterial Culture Stocks.* This study used the bacteria *Actinomyces* spp from the Microbiology Laboratory Research Center, Faculty of Dental medicine, Universitas Airlangga. The bacteria were regenerated by transferring the bacteria to Nutrient Agar (NA) which was incubated at 37 °C for 18–24 h [6].
- 2.3. *Preparation of Bacterial Suspension.* The inoculated test bacteria were taken with a sterile ose needle, then suspended in a tube containing 0.9% NaCl solution until the turbidity was the same as the standard turbidity of Mc. Farland 0.5 [7].
- 2.4. *Antibacterial Activity Test.* Antibacterial activity test in this study using the dilution method. The dilution method was carried out by preparing eight sterile tubes; one tube containing a negative control (aqua dest), one tube containing a positive control (chlorhexidine), and six tubes containing various concentrations of red grape ethanol extract and filling each tube with BHI-B and bacterial suspension.

Incubate again for 24 h at 37 °C. Then, take 0.1 ml from each tube, infuse it on nutrient agar media using a spreader, and incubate again for 24 h at 37 °C. Count the number of colonies that grow on nutrient agar media manually, expressed by colony forming units [8].

3 Results and Discussion

After observing and counting the bacteria with five repetitions of the antibacterial activity of the red grape ethanol extract, it was found that at a concentration of 15% to 25%, there was no bacterial growth, as can be seen in Table 1., so that the concentration of 15% was determined as the value of MBC. This indicated that the ethanolic extract of red grapes (*Vitis vinifera variant Red globe*) has antibacterial effectivity as a root canal irrigation material against the growth of *Actinomyces spp.* bacteria in vitro (Figs. 1 and 2).

Table 1. The results of the calculation of the bacterial colony growth of *Actinomyces spp* on agar media with five repetitions

Number of Repetition	Concentration							
	K (+)	K (-)	25%	22,5%	20%	17,5%	15%	12,5%
I	0	186	0	0	0	0	0	12
II	0	164	0	0	0	0	0	11
III	0	172	0	0	0	0	0	13
IV	0	170	0	0	0	0	0	15
V	0	181	0	0	0	0	0	14

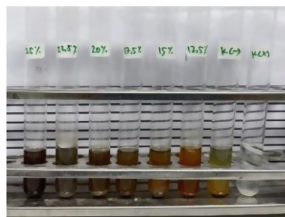


Fig. 1. The results of the solution on the tube liquid medium after incubation for 24 h

The results of the Kruskal Wallis test, a significance value of 0.000 ($p < 0.05$) was obtained, which indicated that there were differences in the effect of red grape (*Vitis vinifera variant Red globe*) ethanol extract at each concentration in inhibiting the growth of *Actinomyces spp.*

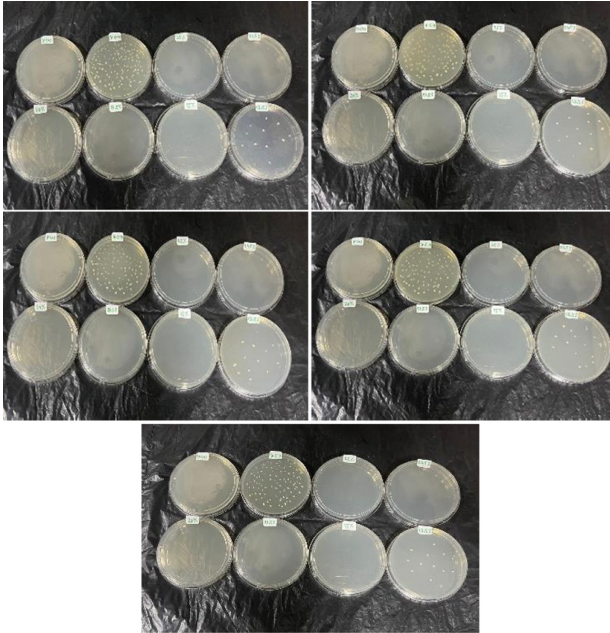


Fig. 2. The results of the growth of *Actinomyces spp.* colonies on agar media with five repetitions

This study showed that the ethanolic extract of red grapes (*Vitis vinifera* variant *Red globe*) has antibacterial effectivity as a root canal irrigation material against the growth of *Actinomyces spp.* bacteria in vitro with minimum bactericidal concentration (MBC) at a concentration of 15%. The research used Kruskal Wallis statistical analysis to determine the difference in the effect of ethanol extract of red grapes (*Vitis vinifera* variant *Red globe*) at each concentration in inhibiting the growth of

Actinomyces spp. bacteria in vitro. The results showed that there were differences in the effect of red grape (*Vitis vinifera* variant *Red globe*) ethanol extract at each concentration in inhibiting the growth of *Actinomyces spp.*, with a significance value of 0.000 ($p < 0.05$).

The ability of the ethanolic extract of red grapes (*Vitis vinifera* variant *Red globe*) to inhibit and kill *Actinomyces spp.* bacteria is due to the active compounds contained in grapes, namely flavonoids, tannins, and phenolic acid [9]. Flavonoids are compounds that are found in many fruits, nuts, and vegetables. Flavonoids have excellent antibacterial activity against gram-positive and gram-negative bacteria because of their ability to damage cell membranes by inhibiting cell membrane function and bacterial energy metabolism [10]. Tannins have antibacterial activity by interfering with the permeability of bacterial cells and the work of bacterial enzymes that play a role in DNA replication

[5]. Phenolic acid has antibacterial properties by crossing the cell membrane by passive diffusion, then disrupting the structure of the cell membrane and increasing the acidity of the cytoplasm causing protein denaturation [11].

4 Conclusion

The ethanolic extract of red grapes (*Vitis vinifera* variant *Red globe*) has antibacterial effectivity as a root canal irrigation material against the growth of *Actinomyces spp.* bacteria in vitro. In addition, using the ethanol extract of red grapes (*Vitis vinifera* Red globe variant) as a root canal irrigation material can support the green economy.

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