Antibacterial effectiveness of moringa leaf (*Moringa oleifera*) nanoparticle paste with different concentration against *Enterococcus faecalis*

Efektivitas antibakteri pasta nanopartikel daun kelor (*Moringa oleifera*) dengan konsentrasi berbeda terhadap Enterococcus faecalis

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ABSTRACT
Background: Endodontic is a treatment that aims to eliminate bacteria from the root canal system. One of the bacteria that causes root canal treatment failure is *Enterococcus faecalis*. Chemomechanical preparation and root canal irrigation are not sufficient to eliminate bacteria completely, so root canal medication is necessary to make it more efficient. Moringa leaves contain tannins, flavonoids and saponins which have antibacterial properties. **Objective**: To determine whether moringa leaf nanoparticle paste had antibacterial effectiveness against the bacterium *E.faecalis*. **Method**: Experimental laboratory with posttest with control group design with *Kirby-Bauer method*. The sample consisted of moringa leaf nanoparticles with concentrations of 1% and 2.5%, calcium hydroxide and aquadest with each of them being repeated 3 times. The diameter of the inhibition zone was determined based on the inhibitory ability of the bacteria saturated on MHA agar media. **Results**: the result showed that the average diameter of the inhibition zone of moringa leaf nanoparticle paste with concentrations of 1% was 8.48 mm, 2.5% was 9.17 mm and calcium hydroxide was 10.2 mm. Data processing showed that the 2.5% moringa leaf nanoparticle paste group had the same antibacterial effect as the calcium hydroxide group. **Conclusion**: Moringa leaf nanoparticle paste (*Moringa oleifera*) with concentrations of 1% and 2.5% was quite effective in inhibiting the growth of *E.faecalis*.
Keywords: nanoparticle paste, moringa leaf, antibacterial, *Enterococcus faecalis*

ABSTRAK
Latar belakang: Endodontik merupakan perawatan yang bertujuan untuk menghilangkan bakteri dari sistem saluran akar. Salah satu bakteri penyebab kegagalan perawatan saluran akar adalah *Enterococcus faecalis*. Preparasi kemomekanik dan irigasi saluran akar tidak cukup untuk menghilangkan bakteri secara tuntas, sehingga perlu dilakukan pengobatan saluran akar agar lebih efisien. Daun kelor mengandung tanin, flavonoid dan saponin yang memiliki sifat antibakteri. **Tujuan**: Untuk mengetahui apakah pasta nanopartikel daun kelor memiliki efektivitas antibakteri terhadap bakteri *E.faecalis.* **Metode**: Sebuah eksperimen laboratorium dengan *posttest with control group design* dengan metode Kirby-Bauer. Sampel terdiri atas nanopartikel daun kelor dengan konsentrasi 1% dan 2,5%, kalsium hidroksida dan akuades yang masing-masing diulang sebanyak 3 kali. Diameter zona hambat ditentukan berdasarkan daya hambat bakteri jenuh pada media agar MHA. **Hasil**: Tampak bahwa diameter rata-rata zona hambat pasta nanopartikel daun kelor dengan konsentrasi 1% adalah 8,48 mm, 2,5% adalah 9,17 mm dan kalsium hidroksida 10,2 mm. Kelompok pasta nanopartikel daun kelor 2,5% menunjukkan efek antibakteri yang sama dengan kelompok kalsium hidroksida. **Simpulan**: Pasta nanopartikel daun kelor (*Moringa oleifera*) dengan konsentrasi 1% dan 2,5% cukup efektif dalam menghambat pertumbuhan bakteri *E.faecalis*.
Kata kunci: pasta nanopartikel, daun kelor, antibakteri, *Enterococcus faecalis*
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INTRODUCTION

Endodontic is a treatment on pulp and periapical tissue that aims to eliminate bacteria and their products from the root canal system so that the teeth can be preserved as long as possible in the oral cavity. Most of them are anaerobic bacteria, one of which is *Enterococcus faecalis* (*E.faecalis*) which causes primary infection.[1] Chemomechanical preparation and root canal irrigation must be accompanied by steps of root canal medication to be more efficient.[2,3]

The most commonly used medicament material today is calcium hydroxide ($Ca(OH)_2$) that has the ability to inactivate bacterial endotoxins and is well accepted by tissues as a root canal medicament. However, calcium hydroxide can cause inflammation during the first few days and dissolves readily. Therefore, the use of natural materials can be used as an alternative.[4]

One of the natural ingredients that have antibacterial properties is moringa leaf (*Moringa oleifera*) because it contains secondary metabolite compounds that have antibacterial activity such as saponins, flavonoids and alkaloids with the ability to damage cell membranes.[5,6] Moringa leaf ethanol extract is able to inhibit bacterial growth. However, in the form of an ordinary extract, this preparation has a weakness, namely its low water solubility, which reduces its bioavailability. So in this study, it was made in the preparation of moringa leaf nanoparticle paste.[7] Nanoparticles are particles measur-

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ing 1-100 nanometers which aim to overcome the solubility of insoluble active substances, improve low bioavailability, modify drug delivery systems, increase stability of active substances and improve absorption. The advantage of nanoparticles is their ability to penetrate the intercellular space that can be penetrated by colloidal particles.[8]

Based on the description above, the authors are interested in conducting research on the antibacterial effectiveness of moringa leaf nanoparticle paste with different concentrations against *E.faecalis* bacteria as an alternative for root canal medicaments.

METHODS

The sample of this study used a paste of moringa leaf nanoparticles which is a species of the monogeneric family *Moringaceae*.[9] The *E.faecalis* bacteria used was ATCC 29212. The bacterial inhibition test used the *Kirby-Bauer method*, which is a diffusion method using disc paper as a medium to absorb antibacterial material which was saturated into the test material and repeated 3 times. The data obtained were analyzed using the *Kruskall Wallis* statistical test.

RESULTS

This study observed the antibacterial effectiveness of moringa leaf nanoparticle paste with different concentrations against *E.faecalis*. The treatment group was divided into 4; aquadest, 1% moringa leaf nanoparticle paste, 2.5% moringa leaf nanoparticle paste and calcium hydroxide. Each group as many as 3 samples.

Table 1 The results of the measurement of the average diameter of the inhibition zone and the standard deviation of *E.faecalis* bacteria

Group	N	Inhibition Zone	
		Mean	± SD
negative control (aquadest)	3	0	± 0
Moringa leaf nanoparticle paste 1%	3	8.48	± 0.45
Moringa leaf nanoparticle paste 2.5%	3	9.17	± 0.16
positive control (calcium hydroxide)	3	10.2	± 0.99

Table 1 shows the difference in the average inhibition zone in each treatment group, it appears that all test groups have inhibition zone values, except for the aquadest test group. The average diameter of the inhibition zone of the positive control test group (calcium hydroxide) showed the largest value compared to the other test groups. The difference in the average diameter of the inhibition zone between concentrations of 1% and 2.5% did not differ much.

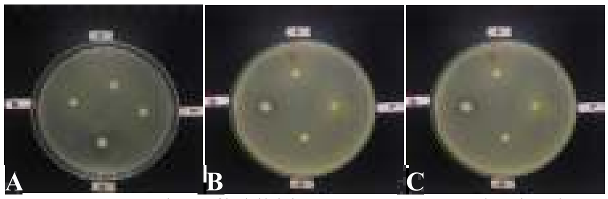

Figure 1 Formation of inhibition zone on MHA that has been incubated for 48 hours; **A** replication 1; **B** replication 2; **C** replication 3.

Table 2 Statistical test results of the zone of inhibition of *E. faecalis* bacteria.

Concentration	Zone of Inhibition	
	Normality test*	Comparison test**
-control (aquadest)	-	
Moringa leaf		
nanoparticle paste 1%	0.042	0.041**
nanoparticle paste 2.5%	0.480*	
+control ($Ca(OH)_2$)	0.738*	

*Shapiro Wilk test: p>0.05; data distribution normal
**Kruskall Wallis: p<0.05; significant

In the observation of the zone of inhibition of the bacterium *E.faecalis*, the *Shaphiro Wilk* statistical test was carried out to determine the normality value and earned $p<0.05$, which means the data was not normally distributed, so the test was continued with the non-parametric test, namely *Kruskall Wallis* (table 2). Based on the *Kruskall Wallis* statistical test, a significance value of 0.000 ($p<0.05$) was obtained, which means that there was a significant difference between the treatment groups. Furthermore, the *Mann Whitney* further difference test was carried out to determine the difference between the 2 variables.

The results of the further difference test of the zone of inhibition between the treatment groups for the *E.faecalis* bacteria showed a significant value on average ($p<0.05$). The whole test group when compared with the aquadest as negative control group had different antibac-

Table 3 *Mann Whitney* statistical test results for the zone of inhibition of *E.faecalis* bacteria.

Control group (i)	Comparison (j)	Zone of Inhibition	
		Mean difference (i-j)	p-value*
1% concentration	2,5% concentration	-0.69	0.050*
	calcium hydroxide	-1.107	0.040*
	aquadest	8.48	0.037*
2,5% concentration	calcium hydroxide	-0.417	0.385
	aquadest	9.17	0.000**
calcium hydroxide	aquadest	10.2	0.000**

**Mann Whitney: p<0.05; significant*
***LSD: p<0.05; significant*

terial effects. Moringa leaf nanoparticle paste with 1% when compared to a 2.5% concentration of moringa leaf nanoparticle paste and calcium hydroxide also did not have the same antibacterial effect. The test group that had the same antibacterial effect was 2,5% moringa leaf nano-particle paste with calcium hydroxide.

DISCUSSION

Root canal treatment is indicated for pulp and periapical tissue that is invaded by both saprophytic and pathogenic bacteria.[10,11] Persistent presence of bacteria after root canal treatment can result in failure of the treatment, one of which is *E.faecalis*, which is the most resistant bacteria found in infected root canals.[12]

This study used a paste of moringa leaf nanoparticles which has an antibacterial effect because it contains several phytochemical compounds such as saponins, flavonoids, and alkaloids.[13] Based on the criteria for antibacterial power according to Davis and Stout *cit* Utomo[14] the results of the inhibitory power of moringa leaf nanoparticle paste against *E.faecalis* bacteria showed that concentrations of 1% and 2.5% were classified as moderate with an inhibitory zone 8.48 mm and 9.17 mm (table 1), while calcium hydroxide was classified as strong with an inhibition zone of 10.2 mm (table 1).

According to Sopandani *et al*, who tested the phytochemical content of moringa leaves showed that the extract contains alkaloids, saponins, tannins and flavonoids, so that the extract has shown that they have antibacterial activity against *E.faecalis*.[13] The antibacterial effect of saponins works by interfering with permeability bacterial cell wall. Flavonoid are bacteriostatic which inhibit the use of oxygen by bacteria so that energy metabolism is inhibited. The antibacterial mechanism of alkaloid compounds is to interfere with the peptidoglycan constituent components in bacterial cells so that the cell wall layer is not fully formed and causes cell death.

In this study, it was shown that diameter of the inhibition zone of moringa leaf nanoparticle paste was smaller than that of calcium hydroxide. This happens because calcium hydroxide has good antibacterial properties with a broad spectrum. When applied, this material will decompose into Ca^{2+} ions and OH^- ions which can infect bacteria with their basic nature and make the bacteria unable to survive.[15]

Within the limitations of this study, it was proven that the antibacterial effect of moringa leaf nanoparticle paste was smaller than that of calcium hydroxide. However, the 1% and 2.5% paste were effective and quite good for using as an antibacterial, the 2.5% had a better effectiveness value than the concentration of 1%.

It is concluded that moringa leaf nanoparticle paste with concentrations of 1% and 2.5% was quite effective in inhibiting the growth of *E.faecalis* bacteria.

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