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Antibacterial Effectiveness of N-hexane Fraction Basil Leaves (*Ocimum basilicum*) against *Streptococcus mutans* ATCC 25175 as Archwire Sterilization Compared to Chlorhexidine

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Abstract: ***Archwire is a fixed orthodontic component that can be reused between visits by the same patient, therefore archwire requires sterilization before being reinserted into the patient's oral cavity because the archwire is found to increase the number of Streptococcus mutans, which is considered the main cause of caries and periodontal disease. Basil leaves (Ocimumbasilicum) is an herbal plant that is proven to have antibacterial effectiveness against several types of bacteria. Objectives: Determining the antibacterial effectiveness of n-hexane fraction of basil leaves and chlorhexidine to Streptococcus mutans, and determining the difference in the decrease number of Streptococcus mutans colonies on sterilized Cu-NiTi archwire using basil leaves versus chlorhexidine. Materials and Methods: Test the inhibition zones, MIC (Minimum Inhibitory Concentrations), MBC (Minimum Bactericid Concentration) for antibacterial effectiveness, and decreased number of bacterial colonies Streptococcus mutanson sterilized archwire using basil leaves and chlorhexidine. Results: The n-hexane fraction of basil leaves and chlorhexidine has a inhibition zones, MIC, and MBC. Decreased number of Streptococcus mutans colonies on sterilized Cu-NiTi archwire using the basil leaves with chlorhexidine showed insignificant results. Conclusions: The n-hexane fraction of basil leaves have antibacterial effectiveness against Streptococcus mutans. The n-hexane fraction of basil leaves has the same effectiveness as sterilization material with chlorhexidine.***

Keywords: The n-hexane fraction of basil leaves (*Ocimum basilicum*), *Streptococcus mutans*, antibacterial activities, archwire

1. Introduction

The use of orthodontic devices increases the risk of oral diseases, because the number of bacteria increases due to the form of orthodontic device components that provide a good new surface for microorganisms to accumulate and multiply. [1, 2]Normal flora in the oral cavity include *Streptococcus mutans*, in certain circumstances can turn into pathogens due to predisposing factors. Orthodontic treatment is one of the predisposing factors, because since the orthodontic device is inserted, *Streptococcus mutans* will increase. Plaque that accumulates on the surface of the teeth, due to an increase in the number of pathogenic bacteria *Streptococcus mutans*, is considered a major cause of caries and periodontal disease. [3–5]

A fixed orthodontic component, some used several times during a visit, for example archwire. In a study of patients treated using fixed orthodontics, the number of *Streptococcus mutans* found on stainless steel archwire, NiTi, Cu-NiTi, uncoated archwire and coated archwire has increased. Archwire requires sterilization to remove *Streptococcus mutans* that is on its surface before being reinserted. *Streptococcus mutans* ATCC 25175 is identical to *Streptococcus mutans*which is commonly found on archwire. *Streptococcus mutans* attachment to archwire in the form of adhesion and retention. Research shows that archwire Cu-NiTi, has the highest adhesion and retention of *Streptococcus mutans* compared to other archwire. [3, 6–11]

Chlorhexidine is a chemical sterilizer and is used as an antibacterial "gold standard" in the oral cavity. Chlorhexidine has the disadvantages of causing stain, changing the archwire's mechanical properties, causing corrosion, and is a chemical. [12–14]

Lack of chlorhexidine makes us need alternative natural antibacterial ingredients. Antibacterial material that is environmentally friendly, not carcinogenic, inexpensive, and is an ingredient in the environment around us. One of the natural antibacterial ingredients that has been proven to be a good antibacterial agent against several types of bacteria is basil (*Ocimum basilicum*). Basil (*Ocimum basilicum*) contains fractions of methanol, ethyl acetate, and n-hexane which have antibacterial effects. This fraction has been investigated as an antibacterial in more than 55 bacteria, but not yet on *Streptococcus mutans*. [15, 16]

Based on the above data, researchers are interested in examining the antibacterial effectiveness of n-hexane fraction basil leaves (*Ocimum basilicum*) and chlorhexidine against *Streptococcus mutans* ATCC 25175, and the effectiveness of the n-hexane fraction of basil leaves (*Ocimum basilicum*) as an archwire sterilizer induced by *Streptococcus mutans* ATCC 25175 compared to chlorhexidine.

The research purpose for knowing the inhibition zones, MIC (Minimum Inhibitory Concentrations), and MBC (Minimum Bactericid Concentration) of n-hexane fraction of basil leaves (*Ocimum basilicum*) and chlorhexidine against

Streptococcus mutans ATCC 25175. Knowing the difference in the decrease number of *Streptococcus mutans* ATCC 25175 colonies on Cu-NiTi archwire that was sterilized using the n-hexane fraction of basil leaves (*Ocimum basilicum)* compared to chlorhexidine.

2. Materials and Methods

1) Preparation of *Streptococcus mutans* ATCC 25175
   Streptococcus mutans ATCC 25175 bacterial culture was taken as much as one ose and put into a sterilized medium by etching bacteria on the surface of the media, then incubated at 37 ° C for 48 hours.
2) Determination of Inhibition Zones
   The method used is the diffusion method, where one ose culture of *Streptococcus mutans* is suspended into a sterile physiological NaCl solution up to the equivalent of a Mc Farland solution 0.5 (3x108 cells / ml sample), compared to the standard. *Streptococcus mutans* sensitivity test against n-hexane fraction of basil leaves (*Ocimum basilicum*) and chloerhexidine was carried out on Mueller-Hinton agar medium supplemented with 1ml of *Streptococcus mutans* suspension. Paper discs that have been dropped with n-hexane fraction of basil leaves (*Ocimum basilicum*) and chlorhexidine are placed aseptically on the surface of the agar medium. Inhibition zone diameters were measured after incubation at 37 ° C for 48 hours.
3) Determination of MIC (Minimum Inhibitory Concentrations)
   The method used in determining the MIC (Minimum Inhibitory Concentration) is a dilution method. The test was carried out using a 96 well microplate (Figure 1). Scans were performed using a microplate reader at a wavelength of 620 nm.
4) Determination of MBC (Minimum Bactericid Concentration)
   MBC values were obtained after growing *Streptococcus mutans* in a 96 well microplate solution in lines 5 and 6 of agar media, incubated at 37 ° C for 48 hours, then the bacterial growth was observed. MBC value is determined based on minimal concentrations where no *Streptococcus mutans* is growing.
5) Determination of decreased number of *Streptococcus mutans* colonies on archwire
   Tests on 0.016x0.022 inch rectangular Cu-NiTi arhwire were performed after the MIC and MBC values were obtained in the n-hexane fraction. Testing is done to compare the decrease in the number of colonies, using the TPC (Total Plate Count) method. The *Streptococcus mutans* ATCC 25175 culture was suspended into the BHI broth to the equivalent of a Mc Farland solution of 0.5 (3x108 cells/ml sample) compared to the standard. Archwire Cu-NiTi rectangular 0.016x0.022 inches dipped in the solution for 1 hour, in an incubator. Archwire Cu-NiTi rectangular 0.016x0.022 inches was removed then put into a mixture of BHI and fraction of n-hexane basil (*Ocimum basilicum*) according to MIC with a ratio of 1: 1 for 1 minute. Archwire Cu-NiTi rectangular 0.016x0.022 inches was removed, then the mixture was taken and put into a microplate to be diluted, then planted on agar media, and incubated for 48 hours, then the colony was counted. The same thing is done by using chlorhexidine as an antibacterial agent.
6) Analysis: Statistical analysis using paired T-test with a confidence level of 95% ($p < 0.05$).

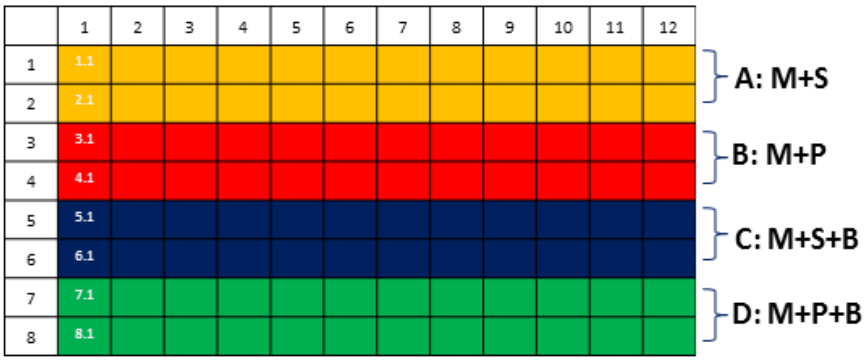


Figure 1: Layout microplate 96 well. M: media, S: sample, P: solvent, B: bacteria. (A) Media and samples. (B) Media and solvents. (C) Media, samples, and bacteria. (D) Media, solvents, and bacteria

3. Results

Table 1: Inhibition zones of N-hexane fraction of basil leaves (*Ocimum basilicum*) and chlorhexidine against *Streptococcus mutans* ATCC 25175

Sample	*Concentration*	*Inhibition Zones (mm)*		*Average (mm)*
		1	2	
N-heksana	1 %	0	0	0
	2 %	0	0	0
	3 %	0	0	0
	4 %	8, 4	8, 1	8, 25
	5 %	9, 1	9, 7	9, 4
Chlorhexidine	2 %	10, 8	10, 4	10, 6

The results in table 1 show that the average value of inhibition zones of n-hexane fraction of basil leaves (*Ocimum basilicum*) against *Streptococcus mutans* ATCC 25175 at a concentration of 4% was 8.25 mm, and at a concentration of 5% it was 9.40 mm. The average value of chlorhexidine inhibition zone against S*treptococcus mutans* ATCC 25175 at a concentration of 2% was 10.6 mm.

Table 2: MIC (Minimum Inhibitory Concentration) N-hexane fraction of Basil (*Ocimum basilicum*) against *Streptococcus mutans* ATCC 25175

ppm	50000	25000	12500	6250	3125	1562.5	781.2	390.6	195.3	97.6	48.8	24.4
MS	0.206	0.368	0.243	0.292	0.231	0.174	0.139	0.155	0.108	0.091	0.087	0.082
	0.287	0.486	0.303	0.231	0.253	0.174	0.156	0.127	0.106	0.093	0.084	0.077
MP	0.053	0.060	0.064	0.067	0.070	0.070	0.071	0.076	0.075	0.074	0.075	0.076
	0.050	0.060	0.062	0.065	0.064	0.066	0.066	0.068	0.072	0.070	0.068	0.070
MSB	0.740	0.592	0.389	0.373	0.446	0.570	0.645	0.657	0.713	0.772	0.766	0.658
	0.627	0.547	0.369	0.364	0.367	0.586	0.584	0.531	0.642	0.631	0.737	0.723
MPB	0.060	0.073	0.076	0.086	0.257	0.432	0.431	0.535	0.699	0.715	0.655	0.699
	0.060	0.074	0.075	0.084	0.279	0.426	0.477	0.464	0.664	0.691	0.507	0.604

Table 2 shows the MIC (Minimum Inhibitory Concentration) of n-hexane fraction of basil leaves (*Ocimum basilicum*) against *Streptococcus mutans* ATCC 25175 at a concentration of 3125 ppm.

Table 3: MIC (Minimum Inhibitory Concentration) of Chlorhexidine against *Streptococcus mutans* ATCC 25175

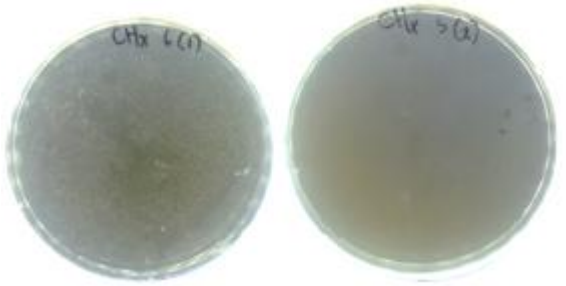
ppm	100	50	25	12.5	6.25	3.125	1.5625	0.78125	0.390625	0.195313	0.097656	0.048828
MS	0.070	0.067	0.073	0.075	0.078	0.079	0.080	0.080	0.079	0.079	0.082	0.080
	0.069	0.067	0.066	0.069	0.069	0.071	0.071	0.076	0.073	0.071	0.073	0.070
MP	0.060	0.064	0.069	0.073	0.076	0.075	0.074	0.078	0.076	0.077	0.077	0.078
	0.059	0.063	0.065	0.067	0.068	0.070	0.069	0.071	0.070	0.070	0.071	0.210
MSB	0.129	0.096	0.095	0.087	0.088	0.091	0.845	0.665	0.760	0.800	0.853	0.722
	0.129	0.112	0.104	0.092	0.095	0.090	0.708	0.654	0.721	0.766	0.656	0.567
MPB	0.378	0.482	0.576	0.606	0.673	0.691	0.765	0.754	0.736	0.778	0.854	0.737
	0.363	0.537	0.537	0.543	0.593	0.770	0.711	0.633	0.697	0.793	0.386	0.685

Table 3 shows the MIC (Minimum Inhibitory Concentration) of chlorhexidine against *Streptococcus mutans* ATCC 25175 at a concentration of 3.125 ppm.

(A) (B)

Figure 2: MBC test plate n-hexane fraction of basil leaves (*Ocimum basilicum*) against *Streptococcus mutans* ATCC 25175. (A) The fraction of n-hexane at a concentration of 3125 ppm, *Streptococcus mutans* is very little. (B) N-hexane fraction at a concentration of 6250 ppm, *Streptococcus mutans* is not seen at all.

Figure 2 shows the results of testing the n-hexane fraction of basil leave (*Ocimum basilicum*) at a concentration of 6250 ppm that no growth of the *Streptococcus mutans* ATCC 25175 was seen. MBC(Minimum Bactericid Concentration)n-hexane fraction of basil leaves (*Ocimum basilicum*)at a concentration of 6250 ppm.



(A) (B)

Figure 3: MBC (Minimum Bactericid Concentration) Chlorhexidine against *Streptococcus mutans* ATCC 25175. (A) Chlorhexidine at a concentration of 3.125 ppm, *Streptococcus mutans* is very little. (B) Chlorhexidine at a concentration of 6.25 ppm, *Streptococcus mutans* is not seen at all.

Figure 3 shows the results of chlorhexidine testing at a concentration of 6.25 ppm that no growth of the *Streptococcus mutans* ATCC 25175 was seen. MBC (Minimum Bactericid Concentration) chlorhexidine at a concentration of 6.25 ppm.

Table 4: Results of t-test analysis of the decrease in the number of *Streptococcus mutans* ATCC 25175 colonies on sterilized Cu-NiTi archwire using the n-hexane fraction of basil leaves (*Ocimum basilicum*) compared to chlorhexidine.

	Average	*P-value*	*Test*
N-heksana	78, 98	0, 089680381*	Non-Significant
Chlorhexidine	95, 00		

Note: * p-value <0.05

The results in table 4 show the average value of the decrease in the number of *Streptococcus mutans* ATCC 25175 colonies on a 0.016x0.022-inch rectangular Cu-NiTi archwire sterilized using the n-hexane fraction of basil leaves (*Ocimum basilicum*) was 78.98, while the average value of the decrease in the number of *Streptococcus mutans* colonies on a 0.016x0.022-inch rectangular Cu-NiTi archwire sterilized using chlorhexidine was 95. Based on the results of the t-test statistical analysis it was concluded that there was no significant difference in the number of *Streptococcus mutans* colonies on a 0.016x0.022-inch rectangular Cu-NiTi archwire that was sterilized using the n-hexane fraction of basil leaves (*Ocimum basilicum*)with those sterilized using chlorhexidine.

4. Discussion

The initial phase of the study was to test the inhibition zone of the n-hexane fraction of basil leaves (*Ocimum basilicum*) and chlorhexidine against *Streptococcus mutans* ATCC 25175 using the disk diffusion method. Inhibition zones of n-hexane fraction of basil leaves (*Ocimum basilicum*) were

carried out at five concentrations, while at chlorhexidine at one concentration. Table 1 shows the inhibition zone in the n-hexane fraction of basil leaves *(Ocimum basilicum*) concentration of 4% with an average inhibition zone of 8.25 mm and at a concentration of 5%, with an average inhibition zone of 9.4 mm. Inhibition zones at 2% chlorhexidine concentration had an average of 10.6 mm. The inhibition zone test results show that the n-hexane fraction of basil leaves (*Ocimum basilicum*) has a fairly good antibacterial power because it is almost as large as the chlorhexidine inhibition zone which is the "Gold Standard". This means that the n-hexane fraction of basil leaves (*Ocimum basilicum*) can inhibit the growth of *Streptococcus mutans*, concluded that the n-hexane fraction of basil leaves (*Ocimum basilicum*) has antibacterial power against *Streptococcus mutans* ATCC 25175.

Testing of MIC (Minimum Inhibitory Concentration) in the n-hexane fraction of basil leaves (*Ocimum basilicum*) and chlorhexidine against *Streptococcus mutans* ATCC 25175 can be seen in table 2 and table 3, showing that the MIC of the n-hexane fraction of basil leaves (*Ocimum basilicum*) at a concentration of 31255 ppm. MIC chlorhexidine at a concentration of 3.125 ppm.

Test of MBC (Minimum Bactericid Concentration) the n-hexane fraction of basil leaves (*Ocimum basilicum*) against *Streptococcus mutans* ATCC 25175 can be seen in figure 2, showing that the MBC n-hexane fraction of basil leaves (*Ocimum basilicum*) at a concentration of 6250 ppm. Figure 3 shows that MBC chlorhexidine against *Streptococcus mutans* ATCC 25175 at a concentration of 6.25 ppm.

The results of inhibition zone test, MIC, and MBC in the n-hexane fraction of basil leaves (*Ocimum basilicum*) and chlorhexidine against *Streptococcus mutans* ATCC 25175, showed that the n-hexane fraction of basil leaves (*Ocimum basilicum*) and chlorhexidine had antibacterial properties against *Streptococcus mutans* ATCC 25175. The concentration of n-hexane fraction of basil leaves (*Ocimum basilicum*) in inhibition zone test, MIC and MBC was greater than the concentration of chlorhexidine to get good antibacterial effectiveness. The n-hexane fraction of basil leaves (*Ocimum basilicum*) is a natural ingredient, so it is not a concern if we use high concentrations in the n-hexane fraction of basil leaves (*Ocimum basilicum*) in its use. [17–19]

Results of phytochemical test n-hexane fraction of basil leaves (*Ocimum basilicum*) contain tannins, saponins, alkaloids, flavonoids, steroids, terpenoids, and phenolics. The presence of flavanoid, phenolic, and terpenoids which provide antibacterial properties. Flavonoids with their antioxidant activity damage bacterial cell walls, inhibit the formation of cell walls, and inhibit the formation of bacterial nucleic acids. Saponin is an antiseptic compound and has an antibacterial effect. Saponins reduce the surface tension of bacterial cell membranes by acting on the phospholipid phosphate group of cell membranes. Saponins enter cells and denaturate cell proteins so that bacterial cells swell and rupture. The phenolic content interacts with the cell biomembrane, passes through the cell membrane, penetrates the cell and destroys the cell. Terpenoids affect the permeability and physiological functions of bacteria. This shows that the n-hexane fraction of basil leaves (*Ocimum basilicum*) contains important compounds that can inhibit bacterial growth, cause cell membrane damage and make it permeable which causes leakage of ions and molecules. Alkaloids inhibit bacterial growth by interfering with the process of bacterial cell division. Alkaloids also damage cell walls and membranes and inhibit the formation of proteins and bacterial DNA. The results of the phytochemical test prove that indeed the phytochemical content of the n-hexane fraction of basil leaves (*Ocimum basilicum*) contains ingredients that can work as antibacterial agents. [20–22]

Tests for the reduction in the number of *Streptococcus mutans* ATCC 25175 colonies on a 0.016x0.022-inch rectangular Cu-NiTi archwire sterilized using n-hexane fraction of basil leaves (*Ocimum basilicum*) with chlorhexidine can be seen in table 4, showed that there was a decrease in the number of *Streptococcus mutans* ATCC 25175 colonies on sterilized Cu-NiTi archwire using n-hexane fraction of basil leaves (*Ocimum basilicum*) with an average decrease of 78.98 colonies, as well as those using chlorhexidine there was an average decrease in 95 colonies. There was no significant difference between the decrease in colony on sterilization using the n-hexane fraction of basil leaves (*Ocimum basilicum)* and chlorhexidine, these results indicate that the n-hexane fraction of basil leaves (*Ocimum basilicum*) has the ability to sterilize Cu-NiTi archwire as well as the sterilization ability using chlorhexidine.

The selection of 0.016x0.022 inch rectangular Cu-NiTi archwire as a sterilization test material, because it has the highest adhesion and retention of *Streptococcus mutans* compared to the other archwire based on material, size, and shape. Based on the material, the adhesion of *Streptococcus mutans* to Cu-Niti archwire is greater than that of NiTi and stainless steel archwire. Based on the shape, the adhesion of *Streptococcus mutans* to arhwire with rectangular shape is greater than that of round and square. The Cu-NiTi archwire surface roughness is the greatest. [3, 8, 11, 23]

Archwire Cu-NiTi has a composistion 42.99% titanium; nickel 49.87%; chromium 0.50%; and copper 5.64%. Copper has antimicrobial power, with a mechanism causing cell membranes to rupture which causes loss of membrane potential and cytoplasmic content, copper ions induce the formation of reactive oxygen which causes further cell damage, DNA genes and plasma are destroyed. Copper antimicrobial power in Cu-Niti archwire does not occur because the copper content is very small, only 5.64%. [24, 25]

The results of the reduction in the number of *Streptococcus mutans* ATCC 25175 colonies on a 0.016x0.022-inch rectangular Cu-NiTi archwire, which were sterilized using the n-hexane fraction of basil leaves (*Ocimum basilicum*) as good as the results of sterilization using chlorhexidine. Based on the explanation above, 0.016x0.022 inch rectangular Cu-NiTi archwire has the highest *Streptococcus mutans* adhesion and retention level compared to other archwire. It was concluded that the n-hexane fraction of basil leave (*Ocimum basilicum*) has antibacterial effectiveness which can then be used as an archwire sterilizing agent, because n-hexane fraction of basil leave (*Ocimum basilicum*) can reduce the number of

Streptococcus mutans colonies which have the highest *Streptococcus mutans*.

5. Conclusion

The n-hexane fraction of basil leaves (*Ocimumbasilicum*) and chlorhxidine has antibacterial effectiveness against *Streptococcus mutans* ATCC 25175. The n-hexane fraction of basil leaves (*Ocimumbasilicum*) has the same effectiveness as a sterilizing agent with chlorhexidine.

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