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Research Report

The effectiveness of mimba oil (*Azadirachta indica A. Juss*) spray disinfectant on alginate impression

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ABSTRACT

Background: *Alginate impression contaminated by saliva and blood could potentially cause cross contamination. To prevent this, the impression has to be disinfected by disinfectant liquid, such as mimba oil. Mimba oil (Azadirachta indica A.Juss) has some chemical content, such as azadirachtin, which is a phenol group used as antibacterial and antimalaria, nimbolide used as antibacterial and antimalarial, and nimbidin used as antibacterial and antifungal.* ***Purpose:*** *The purpose of this study was to find out the most effective concentration of mimba oil as disinfectant to decrease microorganism colony on alginate impression.* ***Methods:*** *Thirty six samples were taken from 9 respondents. This alginate impression was divided into 4 groups: group 1 sprayed with sterile aquadest (as control group), group 2 sprayed with mimba oil 50% for 30 seconds, group 3 sprayed with mimba oil 75% for 30 seconds, group 4 sprayed with mimba oil 100% for 30 seconds. The microorganism colony was counted by colony counter. The sample data then were analyzed with Kolmogorov-Smirnov test, and was tested with Kruskal Wallis test and Mann Whitney test for further analysis.* ***Results:*** *There was significant difference among each group, $p = 0.01$ ($p < 0.05$).* ***Conclusion:*** *In conclusion, usage of 50% concentration of mimba oil as disinfectant is effective to decrease microorganism colony on alginate impression.*

Key words: *Alginate impressions, mimba oil, oral microorganism*

ABSTRAK

Latar belakang: *Cetakan alginat yang terkontaminasi saliva dan darah dapat berpotensi terjadinya infeksi silang. Untuk mencegah hal tersebut, cetakan didisinfeksi dengan bahan disinfektan cair seperti minyak mimba. Minyak mimba (Azadirachta indica A.Juss) memiliki beberapa kandungan kimia, antara lain Azadirachtin yang merupakan kelompok fenol yang memmiliki efek antibakteri dan antimalaria, nimbolide memiliki efek antibakteri dan antimalaria sedangkan nimbidin memiliki efek antibakteri dan antijamur.* ***Tujuan:*** *Tujuan dari penelitian ini adalah untuk menentukan konsentrasi yang paling efektif pada minyak mimba sebagai disinfektan untuk mengurangi jumlah koloni mikroorganisme pada cetakan alginat.* ***Metode:*** *Tiga puluh enam sampel diambil dari sembilan subyek. Cetakan alginat dibagi menjadi empat kelompok: kelompok 1 disemprot dengan aquades steril (kontrol), kelompok 2 disemprot dengan minyak mimba 50% selama 30 detik, kelompok 3 disemprot dengan minyak mimba 75% selama 30 detik, kelompok 4 disemprot dengan minyak mimba 100% selama 30 detik, Jumlah koloni mikroorganisme dihitung menggunakan colony counter. Data dianalisa dengan uji Kolmogorov-Smirnov, dilanjutkan dengan uji Kruskal Wallis dan Mann Whitney.* ***Hasil:*** *Terdapat perbedaan bermakna pada masing-masing kelompok, $p = 0,01$ ($p < 0,05$).* ***Kesimpulan:*** *Dapat disimpulkan dengan konsentrasi 50% sebagai desinfektan cetakan alginate telah efektif menurunkan pertumbuhan microorganisme rongga mulut.*

Kata kunci: *Cetakan alginat, minyak mimba, mikroorganisme rongga mulut*

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INTRODUCTION

In dental care or denture making, oral tissue model is necessary to be made by doing impression with impression material and then fill it with plaster. The mostly used impression material in dentistry is alginate.[1] However, in the impression process, the alginate impression can be contaminated with alginate pathogenic microorganisms causing cross infections, such as pneumonia, tuberculosis, herpes, hepatitis, and AIDS.[2] But, the risk of those cross infections can be reduced by using disinfectant materials.[1]

The disinfection process of the alginate impression can be done by spraying and immersion in disinfectant material since the alginate material can not be sterilized.[3] According to research results, disinfectant spraying technique can have the same antimicrobial activity as immersing one, but it does not affect the dimension stability of the alginate impression. [4] Unfortunately, disinfectant materials sold in markets sometimes are not affordable for public because relatively high price. According to the WHO, traditional herbal medicine can become alternative disinfectant materials because of their easily known ingredients, safer, more efficient, and more easily obtained.[5] Mimba (*Azadirachta indica A. Juss*) can be used to solve tooth and gum problems, ulcers, malaria, typhoid, and gastroenteritis.[6,7]

Mimba seeds contain oil that can be used to inhibit the growth of *Salmonella thyposa* and *Staphylococcus aureus* bacteria at concentrations of 50%, 75% and 100%.[7] It is because mimba oil (*Azadirachta indica A.Juss*) has useful chemical ingredients, such as azadirachtin, nimbolide and nimbidin, which have antibacterial, antifungal and antimalarial effects.[7]

This research is aimed to determine the effectiveness of mimba oil (*Azadirachta indica A.Juss*) spraying with concentrations of 50%, 75% and 100% used as disinfectant to reduce the number of microorganism colonies on alginate impression. If the results of this research show that mimba oil is effective, it can become an alternative disinfectant material for alginate impression and may be developed for other health purposes because it has useful pharmacological characteristics.

MATERIALS AND METHODS

This research was conducted from April to October 2011. The location of the research for the impression process took place in Prosthodontic Clinics of Dentistry Faculty, Airlangga University. Meanwhile, sample treatment process and measuring process of growing microorganism colonies were conducted ini Microbiology Laboratory of Dentistry Faculty, Airlangga University. Qualitative test and mimba oil concentration were conducted in Phytochemistry Pharmakognosis Laboratory of Pharmacy Faculty, Airlangga University.

Mimba oil was qualitatively examined through organoleptis examination, pH determination, specific gravity, and refractive index. The mimba oil was made into certain concentrations, 50%, 75%, and 100%, by using sterile aquadest diluent. Nine subjects with certain criteria, 20–25 year old men with complete and normal dental structure, good oral hygiene, no dental caries and filling, good general health, no antibiotic treatment, no periodontal disorders, and no smoking habit were used in this research.

Alginate impression was made with a 7 grams of alginate powder and 15 ml of water mixed with a rubber spatula in bowl for 30 seconds, and then put into impression tray for 1.5 minutes.[8] Those nine subjects were asked to rinse by using water before alginate was impressed on their upper jaw for 1.5 minutes, and then the impressions were removed from their oral cavity. The alginate impressions were washed with running water for 15 seconds.[8]

Four alginate pieces of their posterior palate were taken by using sterile aluminum ring with thickness of 2 mm and diameter of 10 mm.[9]

Figure 1. Ring with thickness of 2 mm and diameter of 10 mm.

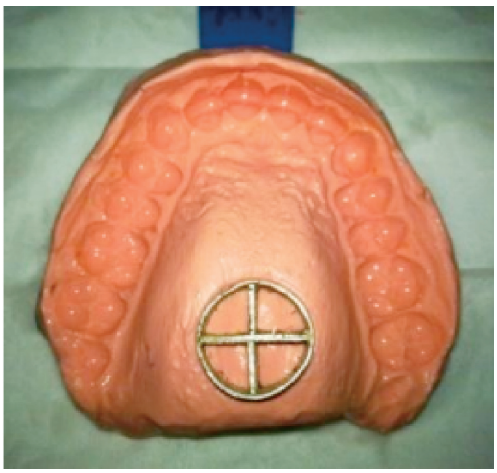

Figure 2. The impression results of the alginate impressions cut by using ring.

The alginate in group I was sprayed with 2 ml of sterile aquadest for 30 seconds as control group, that in group II was sprayed with 2 ml of 50% mimba oil for 30 seconds, that in group III was sprayed with 2 ml of 75% mimba oil for 30 seconds, and that in group IV was sprayed with 2 ml of 100% mimba oil for 30 seconds.[7,8] Once given the treatment, all those groups were then washed with 2 ml of sterile aquadest for 15 seconds. Each of alginate mold piece was inserted into test tubes containing BHI liquid media, and then was thinned, about 10^{-2}. Next, 0.1 ml of BHI liquid media were planted on petridish containing blood agar. It was then incubated for 24 hours at 37° C, and microorganism colonies were counted by using colony counter with CFU/ml units.[8]

RESULTS

Mimba oil obtained was qualitatively tested before being used in this research. The qualitative test results of neem oil can be seen in table 1.

Table 1. Qualitative test result of mimba oil

Type of examination	Examination results	References[10]
Color	Yellow-brown color	Yellow-brown color
Aroma	Strong	Strong like onions
Flavor	Bitter	Bitter
pH	5	5.3
Density	0.918	0.922 g/mL
Refractive index	1.4650	1.4615–1.4705

Based on the data obtained, mimba oil used in this research was in accordance with the description in the reference.[10]

Based on the observation and counting results, the number of microorganism colonies on the alginate impressions sprayed in those 4 groups, namely a control group and 3 treatment groups sprayed with mimba oil for 30 seconds with concentrations of 50%, 75 % and 100% can be seen in table 2.

The average number of microorganism colonies in the control group and those three treatment groups sprayed with 50%, 75% and 100% mimba oil was decreasing (Table 2). In the table above, it is also known that the lowest number of the colonies was in the treatment group sprayed with 100% mimba oil.

Before analysis comparison test among those groups was conducted, normality test had been conducted first on each group by using Kolmogrov-Smirnov test. The result then showed that all those groups had greater p values than 0.05 ($p > 0.05$) indicating that the data on those groups were normally distributed. Next, One-Way ANOVA test was conducted to see the homogeneity and significance of those groups, and in the Levene test, it is known that p value obtained was 0.05 indicating that the data on all those groups were not homogeneous. Since the data were not homogeneous, then non parametric test was conducted by using Kruskal Wallis test. The result then showed that p was 0.05 indicating that there was a significant difference among the four groups.

Table 2. The mean and standard deviation results of the number of microorganism colonies in oral cavities after the alginate impressions sprayed with sterile aquadest and 50%, 75%, and 100% mimba oil

Sample	Mean	Standard Deviation
I	77.33	17.45
II	28.44	7.63
III	1655	4.58
IV	7.22	2.63

Note: I: The alginate impression was sprayed with sterile aquadest (control); II: The alginate impression was sprayed with 50% mimba oil; III: The alginate impression was sprayed with 75% mimba oil; IV: The alginate impression was sprayed with 100% mimba oil

To find out the differences among those treatment groups, Mann Whitney test was conducted (Table 3). Mann Whitney test results between the control group and those groups sprayed with 50%, 75% and 100% mimba oil.

Table 3. The result of Mann-Whitney test between the control group and those three treatment groups with 50%, 75% and 100% mimba oil

	Control	5%	75%	100%
Control	-	0.001*	0.001*	0.001*
50%		-	0.001*	0.001*
75%			-	0.002*
100%				-

Note: * = Significant difference

In each of those comparisons, there was significant difference in the number of microorganism colonies by using Mann Whitney test (Table 3).

DISCUSSION

Samples used were pieces of the alginate impressions of the posterior palate because there was no significant difference between the colonies of microbes growing on tooth region I, P and M so that it can be stated that the number of microbial colonies on the palate was generally more evenly.[11] The alginate impressions of the posterior

palate were then cut by using sterile aluminum ring with thickness of 2 mm and diameter of 10 mm divided into 4 sections.[9] After being taken out from the mouth, the alginate impressions should be washed with water to remove saliva, debris, and blood.[12] Alginate impression materials had reacted with water to form alginate calcium salts precipitating in the forms of woven fiber-like tissue with water molecules in its capillary space.[3] Capillary space and irregular alginate shape facilitated the attachment of microorganisms of oral cavity to the alginate impressions causing the microorganisms in the alginate impressions were not easily lost by washing with water only.

In this research, spraying technique, moreover, was used to disinfect since the technique of disinfectant spraying showed the same antimicrobial activity as that of immersing, but did not affect the dimensional stability of the alginate impressions, such as dimensional accuracy, stability and wettability.[4] And, concentrations of mimba oil used as disinfectant to reduce the number of microorganism colonies on the alginate impressions were about 50%, 75% and 100%. This is because based on a research it is known that neem oil can inhibit the growth of *Salmonella typhosa* at concentration of 50% with resistance area diameter of 3 mm, at concentration of 75% with resistance area diameter of 4 mm, and at concentration of 100% with resistance area diameter of 5 mm. Meanwhile, mimba oil can inhibit the growth of *Staphylococcus aureus* at concentration of 50% with resistance area diameter of 4 mm, at concentration of 75% with resistance area diameter of 5 mm, and at concentration of 100% with resistance area diameter of 6 mm.[7]

Based on the results, furthermore, there was significant difference of the number of microorganism colonies between on the alginate impression sprayed with sterile aquadest and on those sprayed with neem oil used as disinfectant. The average number of microorganism colonies on the alginate impression sprayed with 100% mimba oil was about 7.22. While, the average number of microorganism colonies on the alginate impression sprayed with 75% mimba oil was about 16.55. And, the average number of microorganism colonies on the alginate impression sprayed with mimba oil 50% was about 28.44. In short, it means that the greater the concentration of disinfectant that contact with the alginate impression, the lower the number of microorganisms grows. This is because their power depends on the concentration of antimicroorganism materials, time and temperature. Thus, the greater the concentration of disinfectant is dissolved in water, the more effective the power to inhibit the growth of microorganisms.[13]

In the spraying of 100% mimba oil on the alginate impression, there was still microorganism colony grown since the microorganisms is considered as a kind of microorganisms that cannot be inhibited by mimba oil. That mimba oil can not effectively inhibit the microorganisms is because aaccording to some researches mimba oil is effective to inhibit the growth of several specific types of microorganisms, such as *Streptococcus mutants, Staphylococcus aureus, Salmonella thyphosa, Staphylococcus coagulase, Plasmodium falciparum, Mycobacterium tuberculosa, Tinea rubrum, and Candida albicans*.[6,7,14,15]

The ingredients contained in mimba oil, furthermore, are *Azadirachtin, nimbolide*, and *nimbidin* which have antibacterial, antifungal and antimalarial characters. *Azadirachtin* is a derivative phenol compound which has antibacterial and antimalarial characters that can inhibit the growth of *Streptococcus mutants* and *Plasmodium falciparum*.[6] Phenol can be used as disinfectant because it can damage the cell walls of microorganisms. The cell walls of microorganisms serve to maintain the integrity of the cell with osmotic pressure if the cells are in hypotonic condition in which the concentration of the fluid outside the cell is less than that inside the cell.[16,17]

Besides, another active ingredients of mimba oil is a group of tetranotriterpenes nimbolide which has antibacterial character in the growth of *Plasmodium falciparum malaria*, *Staphylococcus aureus* and *Staphylococcus coagulase*. Meanwhile, a group of tetranotriterpenes nimbidin has antifungal and antibacterial characters in the growth of *Tinea rubrum* and *Mycobacterium tuberculosis*.[14] In conclusion, 50% mimba oil as desinfectan is already effective decreases microorganism colonies in the alginate impression.

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