Thymoquinone Potency in Denture Plaque Hydrolysis In Vitro

Muhammad Luthfi[1]*, Indah Listiana Kriswandini[1], Afrisal Erviyansyah[2]

1. Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.
2. Undergradute Student, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

Abstract

The prolonged accumulated plaque, may lead to microorganism multiplication, thus causing pathological changes in oral cavity. Dental plaque consists of 20%-30% intercelullar matrices and 70% microorganisms. Thymoquinone denotes bio-active agent extracted from plants that has antibacterial and antifungal activity. This study aimed to determine the effective concentration of thymoquinone to detached plaque from acrylic plate. This was an analytical laboratory study involving denture users as sample. The accumulated denture plaque was replicated in BHIB solution. Acrylic plates were immersed in BHIB, and divided into groups for hydrolysis assessment; control group, then were macerated in distilled water; as for treatment group, the plates were macerated in thymoquinone in varying concentration for a range of time periods. Optical density of hydrolysis result analysis was done at a wavelength of 600nm. **Result.** The lowest hydrolysis rate recorded was control group, with mean of 31.32%. Thymoquinone at 50% concentration, with 10 minutes maceration periods showed the highest hydrolysis rate (74.42%). The effective concentration of thymoquinone for denture plaque hydrolysis is 50%, soaked for 10 minutes.

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Introduction

Dental and oral health, is considered as an important aspect in general health. Loss of teeth may give rise to discomfort, affecting daily activity, such as speaking and chewing difficulty, which may lower self confidence. Therefore, loss of teeth may affect quality of life. Untreated caries, periodontal disease and/or trauma may, consequently, lead to tooth loss.[1] Rapid economic development and population growth indicate increase in denture users, the percentage of denture wearer in Indonesia was 4.5% of total population, and14.5% of denture wearers were elderly, aged more than 65 years old.[2]

Elderly, in general, have fewer teeth, and along with the advancing age, the risk of loosing teeth increases.[3] Partial loss of teeth may give rise to both oral and general health problem, which then affecting quality of life, including food savoring and nutritional intake.[4] Besides, tooth loss may impair mastication system, including occlusion, tongue, saliva and muscles. Moreover, most of elderly experience decrease saliva production.[5] Therefore, denture is necessary to improve those conditions.

Denture constitutes one of appliance with plate as a basis that lays on oral soft and hard tissue. The most commonly used material as denture basis is acrylic resin, made of polymethyl methacrylate (PMMA).[6] PMMA has excellent characteristics, such as aesthetically pleasing, since having similar color and texture to the gingiva. Besides, PMMA also has low water absorption and minimal dimensional changes, non toxic, and easy to manipulate.[7] Despite the good qualities, PMMA also has undesirable characteristic, that is residual monomer. Residual monomer may create micro-porosity thus facilitate pellicle and microorganisms adhesion and forming denture plaque.[8]

Microorganisms present in plaque are Streptococcus mutants and some anaerobes like Fusobacterium and actinobacteria.[9] Intercelullar matrices occupy 20-30% of total plaque mass,

Corresponding author:
Muhammad Luthfi
Department of Oral Biology,
Faculty of Dental Medicine, Universitas Airlangga
Surabaya, Indonesia.
E-mail: m.luthfi@fkg.unair.ac.id

which consists of organic and non-organic substances resulting from saliva, gingival crevicular fluid, and bacterial products. Organic matrices such as polysaccharide, protein, glycoprotein, and lipid material. Glycoprotein denotes the major component of pellicle.[10] Prolonged denture plaque accumulation may increase microorganism colony, that allows pathological changes in oral mucosa, such as denture stomatitis, halitosis, and caries.[11]

The commercially available denture cleanser contain active agent such as hypochlorite, peroxide, and enzime.[12] However, those substance may damage denture base, thus, denture base with minimal effect on denture base is required.[13]

Black cumin (*Nigella sativa*) is proven containing bio-active agent that may act as anti-bacteria, anti-fungi, and antioxidant.[14] Black cumin contain three active substances, which facilitate plaque hydrolysis, there are thymoquinone, thymol, and tannin.[15] Thymoquinone has been proven to be effective toward gram positive and negative bacteria. Thymoquinone constitutes terpenoid group that is major compound identified in black cumin essential oil, with content of 7.8-13.7%.[16] Thymoquinone can form irreversible complex with nucleophilic amino acid, and inactivates bacterial protein, and subsequently, lead to lysis.[15] Based on those foregoing background, this study is conducted aiming to find whether thymoquinone is effective in hydrolysing denture plaque. Therefore, this study using thymoquinone at 3.125%, 6.25%, 12.5%, 25%, and 50%.

Materials and methods

This study received approval from an ethical clearance letter from the ethical committee number 223/HRECC/FODM/IX/ 2017. This study was a laboratory-based experimental research with a post test-only control group. Plaque samples were obtained from upper full denture plaque, cultured using Brain Heart Infusion Broth (BHIB) solution. All the respondents involved in this study signed an consent form. Acrylic plate, sized 1x4cm, which were incubated in BHIB solution for 24 hours.

Samples were divided into 6 groups, that are control group and 5 treatment group. After incubated in saliva added with bacteria culture for 5, 10 and 15 minutes, acrylic plates in control group (K) were immersed in 10 ml distilled water, and plates of treatment group were immersed in 10 ml thymoquinone at concentration of 3.125%, 6.25%, 12.5%, 25%, and 50% respectively for group P1, P2, P3, P4, P5 for 5, 10, and 15 minutes. Then, the plates were removed and stored in cooler box to stop the active substance reaction; while the liquid of plaque hydrolysis were processed for optical density reading by means of spektrophotometer UV (SPECTROPHOTOMETER GENESYS 10 UV VIS Garforth, USA). The liquid were vortexed for 2 minutes, and soaked for 15 minutes. Then, the liquid were centrifuged at 3000rpm 5-10º for 10 minutes, and the supernatant were removed respectively. The optical density reading of the supernatant were done at 600nm wavelength. Meanwhile, the residual plaque at the acrylic plates were brushed in 100 ml distilled water, then undergone the same process for optical density reading.

The optical density of both hydrolyzed and residual plaque were added up to get the number of total plaque. Then, the plaque concentration were assumed in Lambert-Beer's Law, by means of the following formula: C ≈ A (C denotes concentration (M), and A denotes absorbance). The concentration of a substance is considered directly proportional to the absorbance from optical density reading, if the absorbance is in range of 0.2-0.8. Thus, the percentage of hydrolyzed plaque were counted using the following formula:

$$\% \text{ Plaque} = \frac{\text{Absorbance of Hydrolyzed Plaque X 100\%}}{\text{Absorbance of Total Plaque}}$$

The acquired data were analyzed using SPSS. Kolmogorov-smirnov test was used to know if the data were normally distributed, and Levene test of varians was used. Then, the data were analyzed using one way ANOVA and Tukey HSD.[17]

Results

The absorbance level of plaque in liquid were analyzed using optical density reading, by means of spektrophotometer UV at 600 nm wavelength. The data were presented in mean and standard deviation.

The percentage of hydrolyzed plaque, both by distilled water and thymoquinone, were normally distributed, yet were not homogeneous. Thus, the comparison test used was Kruskal-Wallis, and showed the value of $p = 0.000$ ($p < 0.05$).

Based on homogenous subsets test, among the immersing durations, there was no significant differences found. A few differences noted between 5 and 10 minutes, while between 5 and 15 minutes, there was less then 4% differences for thymoquinone 25%.

Based on homogenous subsets tests, among different immersing duration, there was no significant differences found. Differences recorded between 5 and 10 minutes was more or less than 5%, while between 5 and 15 minutes was more than 6% for thymoquinone 50%.

Group		Mean ± SD
5 Minutes	Distilled Water	0.22 ± 0.03
	3.125%	0.30 ± 0.04
	6.25%	0.38 ± 0.03
	12.5%	0.45 ± 0.05
	25%	0.49 ± 0.09
	50%	0.53 ± 0.05
10 Minutes	Distilled water	0.26 ± 0.003
	3.125%	0.35 ± 0.02
	6.25%	0.38 ± 0.003
	12.5%	0.48 ± 0.007
	25%	0.50 ± 0.03
	50%	0.58 ± 0.08
15 Minutes	Distilled water	0.29 ± 0.003
	3.125%	0.37 ± 0.004
	6.25%	0.40 ± 0.004
	12.5%	0.41 ± 0.007
	25%	0.51 ± 0.005
	50%	0.58 ± 0.02

Table 1. Mean and Standard Deviation of Hydrolyzed Plaque.

Group	Concentration	P
Control 5 minutes	3,125%	0.155
	6,25%	0.003*
	12,5%	0.000*
	25%	0.000*
	50%	0.000*

Table 4. LSD Test of Each Group in 5 Minutes of Immersing Duration.

Group		Mean ± SD
5 Minutes	Distilled Water	0.49 ± 0.08
	3.125%	0.42 ± 0.09
	6.25%	0.43 ± 0.09
	12.5%	0.35 ± 0.05
	25%	0.31 ± 0.09
	50%	0.23 ± 0.09
10 Minutes	Distilled water	0.48 ± 0.05
	3.125%	0.42 ± 0.08
	6.25%	0.43 ± 0.09
	12.5%	0.35 ± 0.04
	25%	0.31 ± 0.09
	50%	0.18 ± 0.07
15 Minutes	Distilled water	0.49 ± 0.07
	3.125%	0.42 ± 0.08
	6.25%	0.34 ± 0.06
	12.5%	0.31 ± 0.08
	25%	0.26 ± 0.06
	50%	0.18 ± 0.06

Table 2. Mean and Standard Deviation of Residual Plaque.

Group		Mean (%) ± SD
5 Minutes	Distilled Water	31.32 ± 3.77
	3.125%	42.62 ± 4.80
	6.25%	47.37 ± 5.57
	12.5%	56.64 ± 3.74
	25%	58.76 ± 3.57
	50%	59.97 ± 2.81
10 Minutes	Distilled water	35.24 ± 2.43
	3.125%	45.40 ± 5.28
	6.25%	47.47 ± 5.54
	12.5%	57.58 ± 2.84
	25%	66.10 ± 5.29
	50%	72.42 ± 9.09
15 Minutes	Distilled water	37.08 ± 3.90
	3.125%	47.17 ± 5.36
	6.25%	57.68 ± 8.64
	12.5%	57.83 ± 8.50
	25%	74.35 ± 6.82
	50%	76.43 ± 6.69

Table 3. Mean and Standard Deviation of Optical Density (%) of Hydrolyzed Plaque.

Group	Concentration	P
Control 10 minutes	3,125%	0.307
	6,25%	0.081
	12,5%	0.000*
	25%	0.000*
	50%	0.000*

* Denotes significant difference

Table 5. LSD Test of Each Group in 10 Minutes of Immersing Duration.

Group	Concentration	P
Control 15 minutes	3,125%	0.319
	6,25%	0.001*
	12,5%	0.000*
	25%	0.000*
	50%	0.000*

* Denotes significant difference

Table 6. Table 4 LSD Test of Each Group in 15 Minutes of Immersing Duration.

Control Group			
Duration	N	Subset for alpha = 0.05	
		1	2
5 minutes	6	31.32609	
10 minutes	6	35.24349	35.24349
15 minutes	6	37.08970	37.08970
P		0.155	0.081

Table 7. *Homogenous Subsets* Plaque Hydrolysis in Various Immersing Duration of Control Group.

Thymoquinone 3.125%					
Duration	N	Subset for alpha = 0.05			
		1	2	3	4
5 minutes	6	42.62364	42.62364	42.62364	
10 minutes	6		45.40323	45.40323	45.40323
15 minutes	6		47.17502	47.17502	47.17502
P		0.155	0.081	0.111	0.069

Table 8. *Homogenous Subsets* Plaque Hydrolysis in Various Immersing Duration of Thymoquinone 3.125%.

Thymoquinone 6.25%					
Duration	N	Subset for alpha = 0.05			
		1	2	3	4
5 minutes	6	47.37519	47.37519	47.37519	
10 minutes	6	47.47180	47.47180	47.47180	
15 minutes	6		54.41467	54.41467	54.41467
P		0.081	0.111	0.069	0.091

Table 9. *Homogenous Subsets* Plaque Hydrolysis in Various Immersing Duration of Thymoquinone 6.25%.

Kelompok Konsentrasi Zat Thymoquinone 12,5%			
Waktu	N	Subset for alpha = 0.05	
		1	2
5 menit	6	56.64498	56.64498
10 menit	6	57.58290	57.58290
15 menit	6	57.83306	57.83306
Sig.		0.069	0.091

Table 10. *Homogenous Subsets* Plaque Hydrolysis in Various Immersing Duration of Thymoquinone 12.5%.

Thymoquinone 25%				
Duration	N	Subset for alpha = 0.05		
		1	2	3
5 minutes	6	62.35864	62.35864	
10 minutes	6	62.97992	62.97992	
15 minutes	6	66.48575	66.48575	66.48575
P		0.091	0.632	0.342

Table 11. *Homogenous Subsets* Plaque Hydrolysis in Various Immersing Duration of Thymoquinone 25%.

Thymoquinone 50%			
Duration	N	Subset for alpha = 0.05	
		1	2
5 minutes	6	70.82930	70.82930
10 minutes	6		75.96926
15 minutes	6		76.43332
P		0.632	0.342

Table 12. *Homogenous Subsets* Plaque Hydrolysis in Various Immersing Duration of Thymoquinone 50%.

Discussion

Plaque constitutes a group of bacteria colony, bound in organic matrices and attached to tooth surface.[18] Plaque initially formed through the acquired pelicle on tooth surface, followed by bacteria adhesion and proliferation. Pelicle consists of glycoprotein, that is immediately formed after brushing teeth. Bacteria proliferation makes plaque thicker, and the environment inside the plaque becomes anaerob.[19]

The previous research proved that black cumin extract is an effective antibacterial agent. The main active substance contained in black

cumin extract is thymoquinone. This research is aiming to analyze the effectiveness of thymoquinone in hydrolyzing denture plaque.

Based on the result, there was difference in the effectiveness of thymoquinone in hydrolyzing denture plaque, according to immersing duration and concentration. The higher the concentration and the longer the duration, resulted in the more hydrolyzed plaque. This happened because of anti bacterial activity of thymoquinone, that is effective against both gram positive and negative bacteria. Previous studies stated that thymoquinone is an affective antibacteria against *Staphylococcus spp., Streptococcus spp., Salmonella spp., Pseudomonas aeroginosa, Escherichia coli, Shigella flexneri, Klebsiella pneumonia, Mycobacterium phlei, Vibrio parahaemolyticus, Bacillus cereus, Listeria monocytogenes* and showed low activity against *Enterococcus faecalis, Enterococcus faecium* dan *Streptococcus salivarius*.[20,21] Some other research also proved the effectiveness of thymoquinon against fungi, such as *Aspergillus niger*[22] and *Candida albicans*.[23,24,25]

The hydrolysis process by thymoquinon is occurred through the forming of irreversible complex with the nucleophilic amino acid of the proteins, that inactivate the proteins. Thus, the complex may, consequently disrupt bacterial cell wall and affect the adhesive function of bacteria.[26] Besides, thymoquinone also inhibit adeonosin triphosphate (ATP) synthase.[27] Other study also proved the potency of thymoquinone in inhibiting biofilm formation, yet the underlying mechanism remains unknown.[28] Meanwhile, other study suggested that thymoquinon may affect biofilm formation through inhibiting ATP synthase on *Streptococcus mutans*, since ATP synthase inhibition may impair biofilm and acid formation.[29]

Previous study by observing plaque, stated that there was a decrease of pH along with the increase of plaque adhered to the tooth surface, thus increasing the number of bacteria in the plaque.[30] Thymoquinone also disrupt the adhesive ability of the bacteria.[31] One of bacteria colony that will multiply along with the decrease of pH is *Streptococcus mutans*, which is the main cause of dental caries.[32]

Based on the result of this study, thymoquinone at 50% concentration, with immersing duration of 10 minutes showed the most effective result in hydrolyzing denture plaque. The longer immersing duration, the longer interaction period of thymoquinon with the plaque, thus, showing the highest effectiveness.

Conclusion

Thymoquinone is effective to hydrolyze denture plaque at 50% concentration, with immersing duration of 10 minutes.

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