Proceeding Book

The Indonesian Journal of Dental Research

Proceeding of
The International Symposium on Oral and
Dental Sciences

Current Clinical Approaches in The Prevention of
Caries and It's Implication
Advanced Clinical Approaches for The Prevention of
Dental Caries and Implicated Disease

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The Effect of Anacardium Occidentale Leaves Extract with Various Concentrations Used as Denture Cleanser on Streptococcus Mutans Growth

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Abstract

Introduction: Microbial adhesion on dentures surface can cause proliferation on bacteria colony, leads to formation of plague which induce bad smell in mouth and denture stomatitis. There is evidence about denture stomatitis caused by another biofilm species, Streptococcus mutans (S. mutans). This study was aimed to observe efficacy differences on S. mutans growth inhibition of Anacardium occidentale leaves extract between various concentrations of 25%, 50%, and 75% as denture cleanser after 45 min immersing. Materials and methods: Samples were made from heat-cured resin acrylic plates with dimension of 10 x 10 x 1 mm. Heat-cured resin acrylic plates were immersed in Anacardium occidentale leaves extract with concentration of 25%, 50%, and 75% for 45 minutes. Each concentration contained of 8 samples. In control group, heat-cured resin acrylic plates were immersed in distilled water. To observe inhibiting effect of Anacardium occidentale leaves extract on S. mutans growth, spectrophotometer was used. Data were analyzed using one way ANOVA and LSD test. Results: Data analyses using one way ANOVA showed significant differences (p<0.05). In LSD test, significant differences on S. mutans growth inhibition effect of Anacardium occidentale leaves extract between concentration 25%, 50%, and 75% after immersing heat-cured resin acrylic plates for 45 minutes was found. Conclusion: In conclusion, Anacardium occidentale leaves extract in concentration of 75% used to immerse the acrylic plate for 45 minutes effectively inhibited the growth of S. mutans.

Keywords: heat cured acrylic, anacardium occidentale leaves, s.mutans

Introduction

Acrylic dentures surfaces usually host several microorganisms (fungi and bacteria) that are difficult to remove even by vigorous mechanical cleaning or chemical agent [1]. The accumulation of microorganisms may cause denture stomatitis. Microbial plaque on removable dentures covering the palate causes denture stomatitis in up to 69% of denture wearers [2]. Denture stomatitis is a condition commonly found in 35% -50% complete denture users. The prevalence of denture stomatitis on partial denture users is lower than complete denture users, ranges from 10-70% depending on the study of population [3]. Baena-Monroy Tetal suggested that denture stomatitis was not only caused by the presence of *C.albicans*, but also from other biofilm species that may include *Streptococcus mutans* and *Staphylococcus aureus* [4]. Among these microorganisms, *Mutans Streptococci* (MS) are the most outstanding as they initiate the formation of biofilm and facilitate the adhesion of yeasts to the mucosa

and dentures. Poor denture hygiene is therefore a major factor predisposing the patient to the development of denture stomatitis, since it allows biofilm accumulation. Proper hygiene is mandatory to maintain the oral health of denture wearers. Bacteria MS were identified in 57 (74%) of the 77 dentures examined in the study, 75.4% were mono colonized by the *S. mutans* and 24.6% were multi colonized by the *S. mutans* [5]. Beyari showed that 2 months after denture insertion (1 month after denture cleanser use), the mean of *S. mutans* count in the saliva of the patients decreased [6].

Maintaining denture hygiene may reduce the attachment of microorganisms on acrylic denture. Immersing dentures in disinfectant materials is one way to maintain denture hygiene. Disinfectant materials may reduce the number of microorganisms attached to the denture. Denture cleaning is primarily carried out mechanically using a tooth brush and a suitable detergent or a tooth paste or an ultrasonic cleaning machine [7]. However, if the patient is elderly enough to have reduced manual dexterity, then a combination of mechanical and chemical denture cleansing is indicated [2].

Traditional medicines have been widely accepted in almost all countries in the world. According to the WHO, 80% world population use traditional medicine for primary treatment. One of the commonly used medicinal plants is the *Anacardium occidentale* [8]. *Anacardium occidentale* is a tree fruit crop from Brazil. This plant was brought to India by Portuguese sailors 425years ago, then spread to other tropical and subtropical regions such as Bahana, Senegal, Kenya, Madagascar, Mozambique, Sri Lanka, Thailand, Malaysia, the Philippines, and Indonesia. Among his many producing countries, Brazil, Kenya, and India is the major supplier of *Anacardium occidentale* country in the world. *Anacardium occidentale* spread throughout the country wide (Indonesia) with different names (in West Sumatra named jambu erang, in Lampung called Gayu, in West Java named jambu mede, in Central Java and East Java named jambu monyet) [9]. Result of Jaiswal *et al.* study revealed leaves extract of *Anacardium occidentale* contained tannins and phenolics, saponins, flavonoids, alkaloids, steroids and sugars [10].

Ariyani et al. conducted study on inhibition force of Anacardium occidentale leaves on Streptococcus sanguis growth and showed that 0.25% Anacardium occidentale leaves extract had antimicrobial against Streptococcus sanguis [11]. Infuse of Anacardium occidentale leaves extract with concentration ranges from 25%, 50%, and 100% gave different effectiveness on C. albicans growth on denture base acrylic resin plate [12]. In this study, we observed the efficacy of Anacardium occidentale leaves extract as a denture cleanser with concentrations of 25%, 50%, and 75% on S. Mutans growth, after immersing acrylic resin plate in the extract solution for 45 minutes. The results was expected to provide scientific information on the use of Anacardium occidentale leaves extract as acrylic resin denture cleanser in proper quantity and quality, so it can be used as an alternative material for denture cleaning to maintain denture hygiene.

Materials and Methods

The study was designed as experimental research and conducted in the Laboratory of Dental Materials and Technology and Laboratory of Microbiology, Faculty of Dentistry, University of Jember. The materials used in this study were heat-cured acrylic resin, *Anacardium Occidentale* leaves extract, and suspension of *S. mutans*.

Acrylic heat cured specimens were made in 10mm x 10mm x 1mm dimension. All acrylic plates were grouped to be immersed in 25%, 50%, 75% leaves extract of *Anacardium Occidentale* solution or distilled water for 45 minutes. Each group consisted of 8 samples. After immersion, acrylic plates were subsequently rinsed twice with PBS solution, soaked in BHIB, and vibrated for 45 seconds. The sum of *S. mutans* growth was conducted with the spectrometer.

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Results

Growth rate value of *S.mutans* on acrylic resin plate after immersed in 25%, 50%, 75% *Anacardium occidentale* leaves extract or distilled water for 45 minutes can be seen in Table 1. Homogeneity analysis result showed p> 0.05, thus level of diversity of the treatment was similar (homogeneous). Furthermore, normality test showed p> 0.05, proving normal distribution. Based one-way ANOVA analysis result, the value of p is 0.00 (p <0.05), which means there was significant difference on *S. mutans* growth on acrylic resin plate after being immersed in 25%, 50%, 75% *Anacardium occidentale* leaves extract or sterile distilled water as a control, for 45 minutes. Furthermore, LSD test (Table 2) was carried out to assess the differences between groups and showed significant difference on *S. mutans* growth among all concentrations of *Anacardium occidentale* leaves extract.

Table 1. Growth rate value of *S.Mutans* on acrylic resin plate after being immersed in 25%, 50%, and 75% *Anacardium occidentale* leaves extract for 45 minutes

	An	Anacardium occidentale Leaves Extract Concentration				
	25%	50%	75%	Control		
Mean	4.55 x 10 ⁸	4 x 10 ⁸	3.05 x 10 ⁸	5.72 x 10 ⁸		

TABLE 1. LSD TEST RESULT ON S. MUTANS GROWTH ON ACRYLIC RESIN PLATE AFTER BEING IMMERSED IN 25%, 50%, 75% ANACARDIUM OCCIDENTALE LEAVES EXTRACT AND DISTILLED WATER FOR 45 MINUTES

Anacardium occidentale leaves extract concentration	25%	50%	75%	Control
25%		*	*	*
50%	*		*	*
75%	*	*		*
Control	36	*	*	
A				

^{*}Significance p<0.05

Discussion

Monroy et al. suggested S. mutans as important microorganisms on biofilm formation. These microorganisms produce polysaccharides from sugar metabolism which able to support their adhesion and that of yeasts to the oral mucosa and denture surfaces. Microorganism S. mutans are outstanding, as they initiate the formation of biofilm and facilitate the adhesion of yeasts to the mucosa and dentures [4]. Poor denture hygiene is therefore a major factor predisposing the patient to the development of denture stomatitis, since it allows biofilm accumulation. Proper hygiene is mandatory to maintain the oral health of denture wearers [5]. Denture hygiene may be maintained by dentures cleaning. Denture can be cleaned by immersing them into chemicals. This study examined the effect of Anacardium occidentale leaves extracts in various concentrations as denture cleaning agents on the growth of S.mutans. One-way Anova test result (Table 1) showed significant S mutans growth differences on acrylic resin plate after immersed in 25 %, 50%, 75% Anacardium occidentale leaves extract or distilled water for 45 minutes. Results of LSD test (Table 2) showed significant S mutans growth differences (p <0.05) on acrylic plate immersed in all concentrations.

Extracts from Anacardium occidentale leaves are attributed to having differ pharmacological properties such as antitussive, antisyphilitic, diuretic, and cicatrizant. Tannin component of Anacardium occidentale leaves extract is responsible on its anti inflammatory effect [10]. JV Pereira suggested Anacardium Occidentale leaves extracts may have therapeutic use as

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antibacterial agent against oral bacteria and showed its *in vitro* antimicrobial activity on *Streptococcus mitis, Streptococcus mutans,* and *Streptococcus sanguis* [13].

Study conducted by YS Jaiswal *et al.* showed *Anacardium occidentale* leaves extracts contain tannins and phenolics, saponins, flavonoids, alkaloid, steroid, and sugar [10]. Tannin is an astringent. Research by Fithrony showed attachment inhibition, bacteriostatic, and bactericide effect of tannin material in beetle nut on *S.Mutans* after denture immersion [14]. Polyphenolic, flavonoids, and tannins present in the ethanol extract may be responsible for the antibacterial activity. Tannin is known to show the antibacterial activity by microbial proteins precipitation [15]. Several previous studies suggested antimicrobial effect of tannins may be related to their ability to inactivate microbial adhesions and inhibit cell envelope transport proteins and hydrolytic enzymes such as proteases and carbohydrolases [16]. Wahyuningtyas and Indrastuti showed antibacterial force of tannins and flavonoids of neem leaves (*Azadirachta Indica L juss*) extract on *S.mutans* [17].

Anacardium occidentale leaves also contain saponin and alcaloid, beside tanin. Crude alkaloids and saponin extracts from aerial parts of Anabasis articulata were examined for antibacterial and antifungal activity. Saponin extract was active against all assayed bacteria (Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Bacillus subtilis, Pseudomonas aeruginosa, and a fungal strain, Candida albicans) with minimal inhibitory concentration (MIC) values ranging from 0.5 to 1 mg/ml. The results suggested saponin extract of A. articulata possess antibacterial and anticandidal properties, thus can be used as antimicrobial agents on infectious diseases [18]. Flavonoids as antibacterial compounds act by forming complexes with extracellular proteins that disrupt bacterial cell membranes integrity [19].

Increasing concentration of *Anacardium occidentale* leaves extract solution decreased *S. mutans* growth due increased concentration of tannin. Elevated levels of tannins produced anti bacterial force. Denture immersion duration, 45 minutes, considered as a short time, thus possibly done while patient take a shower.

In this study, besides treatment group, control group of distilled water-immersed acrylic resin was prepared. Data analysis showed significant difference between treatment and control group (p<0.05). Data showed *S.mutans* growth increases in line with longer immersion time. Longer immersion duration in distilled water promoted increase number of *S.mutans* growth. Distilled water does not contain any bacteriostatic and bakteriocide chemical compound, so it may not inhibit *S.mutans* growth.

Conclusion

It can be concluded that 25%, 50%, 75% *Anacardium occidentale* leaves extract as acrylic denture cleaning materials influenced *S. mutans* growth. The higher *Anacardium occidentale* leaves extract concentration, the stronger inhibiting force on *S mutans* growth. However, 75% concentration of *Anacardium occidentale* leaves extract was considered as the most effective in inhibiting *S. mutans* growth on acrylic plate which was immersed for 45 minutes.

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