

## Study the effect of some tooth paste On the oral health

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### Abstract

This study was conducted to investigate the effect of toothpastes used for oral health by acting as antimicrobial against biofilm formations. Different types of toothpastes were selected, including those were made of natural materials and others with artificial chemicals using herbal additives. Five tooth pastes (Lacalut®, Paradontax®, Colgate®, Miswak®, Sanino®) were used on microbiota spectrum of spp. Bacteria (s. mutans, s. viridans) obtained by swab plaque accumulated in teeth from different patients over a period of 2021, the exclusion criteria from the investigation were patients receiving concurrent antibiotic treatment for any purpose. This research was conduct in central environmental laboratory at Baghdad University. Each toothpaste is diluted with distilled water to prepare 0.2 and 0.3 concentrations. The estimation of bacterial spectrum was performed in qualitative manner using standard methods for microbiological inoculation tissue plate culture (TPC) were performed (for bacterial presence) and quantitative qualification of the subjects by, Elisa instrument. It was found that toothpaste which contains natural materials is particularly effective in improving oral health and reducing the incidence of gingivitis caused by microbial bacterial presence. This revealed that the naturally occurrence of different groups of organic compounds flavonoids and phenolic acids (esters), are responsible for many of the biological activities against oral bacteria.

**Key words:** Toothpaste, Biofilm, Oral Health

### Introduction

Biofilm formation is a natural scheme with inside the oral environment, but needs to be controlled through normal brushing that permits to supply from progress of caries and periodontal infections (1). Regular toothpaste formulations include combination of fluorides and detergents, to beautify the effectiveness of brushing and hence stopping illnesses (2). The etiology hypothesis, which proposes that the biofilm formation is complicated pathogenic of microorganisms, fewer than twenty are automatically observed in prolonged proportions at periodontal disease sites. These specific infectious bacterial species activate the host's immune and inflammatory responses that then cause bone and moderate tissue destruction (3). Toothpastes enhance oral hygiene with the aid of improving the impact of mechanical scrubbing with a toothbrush and handling over medicinal agent to the oral cavity. In addition, to their anti-caries impact, maximum toothpastes may be widely

categorized as having cosmetic with healthy oral cavity (4) Orally infection by microbial bacteria leads to formation dental plaque which means adhering the bacteria to the soft tissue and aggregate then biofilm surrounding the effective area that leads to plaque of gingival ,the crevice fluid supply all nutrients needed for the continuous rising bacterial growth and infected place that became difficult to treated by antibiotics because the drug used cannot penetrate and achieve the infective site. (5). The consequence cleaning and enhancing agent is to remove any adherent layer on the tooth from backward to forward to reduce the pain additionally, perseverance of giving foam to crash and get rid of the debris, Flavors are possibly the most essential part of toothpaste as of consumer preferences (6). This observation developed out to study the impact of toothpastes used for oral qualification as overall antimicrobial performance in the direction of biofilm formations.

### Materials and Methods

Five tooth pastes (Lacalut®, Paradontax®, Colgate®, Miswak®, Sanino®) were used on two important bacterial pathogens that have developed a complex network of evasion, counter-inhibition, and subjugation in their battle for space and nutrients are spp. Bacteria (s. mutans, s. viridans) obtained by swab plaque accumulated in teeth from different patients over a period of 2021, the exclusion criteria from the investigation were patients receiving concurrent antibiotic treatment for any purpose. This research was conducted in central environmental laboratory at Baghdad University. Each toothpaste is diluted with distilled water to prepare (0.2mg/ml and 0.3mg/ml) concentrations. The estimation of bacterial spectrum was performed in qualitative manner

using standard methods for microbiological inoculation tissue plate

Culture (TPC) were performed (for bacterial presence) and quantitative qualification of the subjects by, Elisa instrument.

As a beginning microbiological culturing was used for determine the effect of the tooth paste for inhibiting (Bacteriostatic) and killing (Bactericidal), the bacteria after addition in tissue plat culture (TPC) to determining the antibacterial effect susceptibility test of these five toothpaste on strains microorganisms in the sample. This includes sequential dilution. Each toothpaste is diluted with sterile pyrogen-free distilled water to give (0.2mg/ml and 0.3mg/ml) all different chemical substances and reagents used have been of analytical grade as illustrated in table (1) as listed on the package.

**Table (1): Ingredients of various Toothpastes for antimicrobial potential**

Toothpastes	Ingredients as listed on package
Lacalut®	Aluminium Hydroxide Aqua, Sorbitol, Hydroxide, Hydrated Silica, Silica, Poloxamer 188, Sodium Lauryl Sulfate, Hydroxyethylcellulose, Aroma, Aluminium Lactate, Titanium Dioxide, Allantoin, Alluminium Fluoride, Chlorhexidine Digluconate, Bisabolol, Sodium Saccharin.
Paradontax®	Stannous fluoride 0.454 % (0.110 % w/v fluoride ion) glycerin, PEG-8, hydrated silica, pentasodium triphosphate, flavor, sodium lauryl sulfate, titanium dioxide, polyacrylic acid, cocamidopropyl betaine, sodium saccharin.
Colgate®	Sodium Fluoride 0.24% (0.15% w/v Fluoride Ion). Purpose: Anticavity. Inactive Ingredients: Sorbitol, Water, Hydrated Silica, PEG-12, Cellulose Gum, Sodium Lauryl Sulfate, Flavor, Sodium Saccharin, Mica, Titanium Dioxide, FD&C Blue 1.
Miswak®	Calcium Carbonate, Sorbitol, Water, Silica, Sodium Lauryl Sulphate, Flavour, Miswak Extract, Cellulose Gum, Carrageenan, Sodium Silicate, PVM/MA Copolymer, Sodium Saccharin, Zinc Gluconate, Sodium Benzoate, Benzyl Alcohol, CI 77891, p-Thymol.
Sanino®	Aqua, Sorbitol, Hydrated Silica, Glycerin, PEG-8, Sodium Lauryl Sulfate, Dicalcium Phosphate Dihydrate, Aroma, Cellulose Gum, CI 77891, Sodium Fluoride, Sodium Saccharin, Sodium Propylparaben, Limonene.

The antimicrobial action of diverse concentrations of the dentifrices was resolute via changed the agar agreeably diffusion method. A pre-prepared bacterial species gathered from different patients of have been taken as a swab from plaque accumulated in teeth after laboratorial preparation of these species (*S. mutans*, *E. coli*) added in the TPC plate using pipette and for reinforcing our experiment we duplicated the species for accurate results The addition is done at special heat contained cabinet with Bunsen burner for better reading and avoiding solution of any unnecessary errors). Then the toothpastes was added in total the addition was 100 microliter of bacteria and 100 microliter of toothpaste solution.

The control was preparing and leave places (lowest point) in the TPC plate for bacterial samples of 200 µl. As a negative and positive control were used for comparison (7). Then micro dilution method was performed in sterile 96-wells microliter plates according to the protocol described previously (8). Different tooth paste concentrations of (0.2mg/ml and 0.3mg/ml) were prepared containing bacterial cells comparable the plate should be washed with water and the washing should be on the sides of the pits that contain the sample then dried using incubator with slightly increased temperature avoid damaging the sample, and this procedure should be repeated three times for accuracy. After drying the sample, it needs to be inhibited so we use methanol as inhibitor and repeat the washing steps which should be added on the sides of the pits and dried again. The plate leaves for about 24hours and notice the contrast of the

stain color, so if the color is darker that means the bacterial invasion is more invasive and vice versa, but for optimal reading we place the plate in the ELISA device that its indication to measure the permeability of the light through the plate and determine the efficacy of the tooth paste solution and wait for digital result obtained from the device.

**Analysis of Data**

Data were analyzed using statistical analysis system- spss (23) to study the effect of different toothpaste on some bacterial isolates. Least significant difference (LSD) was used to compare the significant difference between means were considered significant when  $P \leq 0.05$ .

**Results**

In this study, five tooth pastes (Lacalut®, Paradontax®, Colgate®, Miswak®, Sanino®) were selected to inspect its ability for being as antimicrobial biofilm infections It can be observed in table (2) they are able to assess human healthy through a variety of commercial products. Certain toothpastes show their capacities in controlling the oral biofilm. Among them, (Lacalut®, Colgate®, Miswak®, Sanino®) are the most effective against oral pathogens. The downside of Paradontax® had no significance value against the test organism ( $p < 0.16$ ) compared to all other toothpaste formulations (Lacalut®, Colgate®, Miswak®, Sanino®) were significantly different ( $P = 0.03, 0.04, 0.03, 0.05$ ) which leads to that there were successfully prevention to the biofilm formation on dentine surfaces and inhibit bacterial growth in the surrounding media, suggesting a promising approach to protecting from dental plaque and secondary caries when applied as a dentine coating.

**Table (2): Descriptive study of toothpaste effect on bacterial biofilm infections**

		Sum of Squares	df	Mean Square	F	Sig.
Miswak	Between Groups	7.444	2	3.722	8.816	.003
	Within Groups	6.333	15	.422		
	Total	13.778	17			
Lacalut	Between Groups	21.778	2	10.889	8.522	.003
	Within Groups	19.167	15	1.278		
	Total	40.944	17			

Paradontax	Between Groups	8.290	2	4.145	2.067	.161
	Within Groups	30.075	15	2.005		
	Total	38.365	17			
Colgate	Between Groups	44.343	2	22.172	3.865	.044
	Within Groups	86.041	15	5.736		
	Total	130.385	17			
sanino	Between Groups	4.111	2	2.056	2.984	.051
	Within Groups	10.333	15	.689		
	Total	14.444	17			

Result of table (3) have reported multiple Comparisons of the toothpaste construction that showed maximum efficiency against the test organism, Escherichia coli and Streptococcus (p=0.003), highly significance was (Lacalut®, Miswax®) but the (miswax®) with large scale toward investigator bacteria with all concentration applied (0. 2mg/ml and 0.3) mg/ml while Lacalut®

inhibited only when applied (0. 2mg/ml) concentration Moreover, all the remaining formulations Colgate®, Sanino®) were statistically significant (p=0.04,0. 05) and respectively only when applied (0. 2mg/ml). Conversely (Paradontax®) was not significantly toward investigator bacteria with all concentration applied.

Table(3)Multiple Comparisons of different toothpastes and some bacterial isolated							
Least significant different (LSD) between means							
Dependent Variable	(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Paradontax	0	2	-.66667-	.48043	.171	-1.6312-	.2978
		3	-.44444-	.48043	.359	-1.4090-	.5201
	2	0	.66667	.48043	.171	-.2978-	1.6312
		3	.22222	.48043	.646	-.7423-	1.1867
	3	0	.44444	.48043	.359	-.5201-	1.4090
		2	-.22222-	.48043	.646	-1.1867-	.7423
Lacalut	0	2	-1.444* <sup>a</sup>	.522	.003	-2.49-	-.40-
		3	-.778-	.522	.142	-1.83-	.27
	2	0	1.444* <sup>a</sup>	.522	.003	.40	2.49
		3	.667	.522	.207	-.38-	1.71
	3	0	.778	.522	.142	-.27-	1.83
		2	-.667-	.522	.207	-1.71-	.38

sanino	0	2	-1.44444*	.52197	.051	-2.4923-	-.3965-
		3	-.77778-	.52197	.142	-1.8257-	.2701
	2	0	1.44444*	.52197	.051	.3965	2.4923
		3	.66667	.52197	.207	-.3812-	1.7146
	3	0	.77778	.52197	.142	-.2701-	1.8257
		2	-.66667-	.52197	.207	-1.7146-	.3812
Colgate	0	2	-1.44444*	.52197	0.04	-2.4923-	-.3965-
		3	-.77778-	.52197	.142	-1.8257-	.2701
	2	0	1.44444*	.52197	0.04	.3965	2.4923
		3	.66667	.52197	.207	-.3812-	1.7146
	3	0	.77778	.52197	.142	-.2701-	1.8257
		2	-.66667-	.52197	.207	-1.7146-	.3812
Miswak	0	2	1.00000*	.49336	0.048	-1.7682-	.2127
		3	-2.0000*	.49336	0.003	-.7682-	1.2127
	2	0	1.00000*	.49336	0.048	-.2127-	1.7682
		3	-1.00000*	.49336	0.048	.0095	1.9905
	3	0	-2.0000*	.49336	0.003	-1.2127-	.7682
		2	-1.00000*	.49336	0.048	-1.9905-	-.0095-
*. The mean difference is significant at the 0.05 level.							

Interestingly, figure (1) Indicates the effect of toothpastes on E-coli biofilm with all concentration applied (0. 2mg/ml and 0.3 mg/ml and demonstrate the ability of toothpastes to reduced and inhibited

the growth of biofilm with slightly difference between each other's the preferable result was found with Miswak®). Moreover, figure (2) reported the same finding streptococcusvirdans.

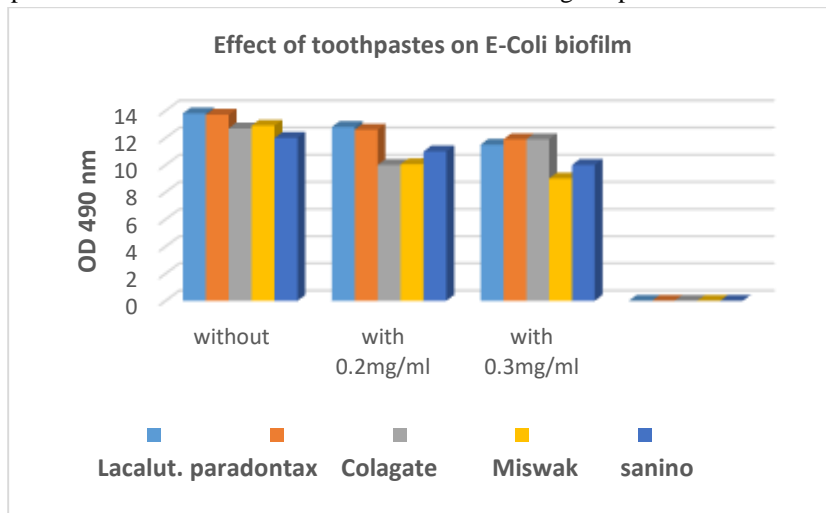


Figure (1): Indicates the effect of toothpastes on E-coli biofilm

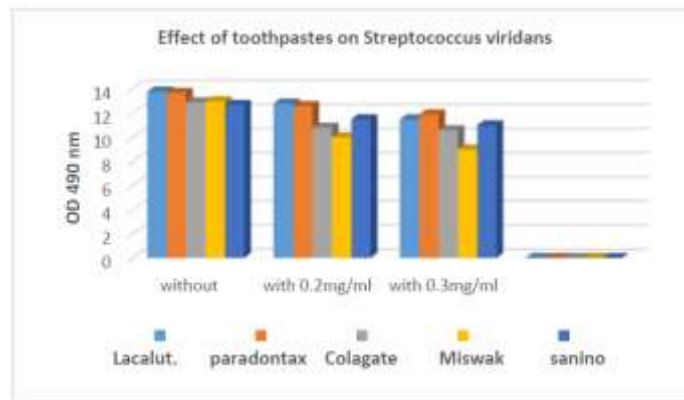


Figure (2): Indicates the effect of toothpastes on streptococcus virdans

### Discussion

Results of table (1) revealed that the five tooth pastes (Lacalut®, Paradontax®, Colgate®, Miswak®, Sanino®) had been able maintained the oral health status by inhibited the formation of biofilm due to the ingredient medicinal compound involved in the formulation structure as illustrated in table (1). This results were in agreement with previous study that suggest the effectiveness of various ingredients in toothpastes to handling and avoiding plaque formation by two points first in assistance cleaning the tooth surface and secondly through providing a therapeutic agent against antimicrobial (10). As we have seen in table (2) only Paradontax® had no significances results against both bacteria This result does not mean the inefficiency of the paste to prevent biofilm formation, but the bacteria selected for the study may be resistant to the composition of the mentioned paste, or it may be due to the dilution factor caused by saliva in addition to the interference of saliva components such as proteins and enzymes on the efficiency of the paste (11). Table (3) designates the great significantly for all the remaining pastes that applied in our study with slightly difference between groups, which confirms the importance of these pastes in eliminating bacteria these findings supported by previous study that supported our results (12). Most of the pastes have a significant value ( $p=0.003$ ) of toothpastes selected in investigator of our results and works to eliminate bacteria in all concentrations (Miswak®) (13). That could be explained due to the formulation

compound inside the structure of pastes which belongs to extract natural Miswak and also to presence of organic flavonoids substances in the internal composition of the paste, which makes it more susceptible to influence on bacteria (14) and in a wider range than the rest of the pastes used in the study and in all concentrations (0.2mg/ml and 0.3mg/ml) that can give excellent results in eliminating bacterial presence. Lacalut®, also has the same significant value ( $p=0.003$ ) as the miswak, but it works only in high concentrations (0.2mg/ml). Finally, the results were plotted to illustrate the effect of the pastes on bacteria and to clarify the difference between pastes group, where it is noted that the preferable paste is the Miswak with high significances results for (*s. mutans*, *s. viridans*) as ant-microbial bacteria as shown in figure (1) and figure (2) These results are identical to what the researchers described as miswak containing natural substances capable of killing oral bacteria and maintaining oral health. (15). We recommend paying attention to oral health because bacterial growth is very fast and penetrates quickly to the soft tissues in the mouth. The bacteria soon become covered with polysaccharides and become a pathogenic system inside. They are constantly fed from the fluids surrounding the tissues and multiply quickly and are difficult to control as using antibiotics treatment because cannot reach the affected area due to difficulty to penetrate the biofilm it is so rigid and what is more dangerous the immune response that became more active and vital in change that can turn into malignant disease.

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## دراسة تأثير بعض معاجين الأسنان على صحة الفم

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### الخلاصة

أجريت هذه الدراسة لمعرفة تأثير معاجين الأسنان المستخدمة على صحة الفم من خلال عملها كمضاد للميكروبات التي تكون الأغشية الجرثومية البكتيرية حيث تم اختيار أنواع مختلفة من معاجين الأسنان، بما في ذلك تلك المصنوعة من مواد طبيعية وأخرى من مواد كيميائية صناعية باستخدام إضافات عشبية.

تم استخدام خمسة معاجين أسنان ( @Sanino/ @Miswak / @Colgate/ @Paradontax / @Lacalut ) على طيف ميكروبات spp. من بكتيريا ( s. mutans , s. viridans ) التي تم الحصول عليها عن طريق أخذ مسحة للمتراكم في الأسنان من مرضى مختلفين خلال فترة 2021 ، كانت معايير الاستبعاد من الدراسة هي المرضى الذين يتلقون علاجاً متزامناً بالمضادات الحيوية لأي غرض من الأغراض. تم إجراء هذا البحث في المختبر البيئي المركزي في جامعة بغداد حيث تم تخفيف كل معجون أسنان بالماء المقطر لتحضير 0.2 و 0.3 من تراكيز المعاجين المستخدمة في الدراسة وتم إجراء تقدير الطيف البكتيري بطريقة نوعية باستخدام الطرق القياسية المتبعة للفحص الميكروبيولوجي (TPC) ( للوجود البكتيري ) ومن ثم القياس الكمي للنتائج بواسطة جهاز الأليزا. وجد أن معجون الأسنان الذي يحتوي على مواد طبيعية يكون فعال بشكل ملحوظ عند مقارنته بالصناعي في تحسين صحة الفم وتقليل حدوث التهاب اللثة الناتج عن الوجود الجرثومي. ويعزى سبب ذلك هو تواجد المركبات الطبيعية الحاوية على مجموعات مختلفة من مركبات الفلافونويد العضوية والأحماض الفينولية (الإسترات) فهي المسؤولة عن العديد من الأنشطة البيولوجية ضد بكتيريا الفم.

الكلمات المفتاحية: معاجين الاسنان، الغشاء الجرثومي البكتيري، صحة الفم