

Original Research Article

EVIDENCE ON ANTIMICROBIAL EFFICACY OF COMMERCIAL TOOTHPASTE

ABSTRACT

This study aims to determine the antimicrobial activity of different brand of commercially available toothpaste in Malaysian outlet, namely Colgate-Total, Mu'min, Tesco and Safi against selected oral microbiome. All the different toothpastes brand were tested for their antimicrobial activity against five oral pathogens namely *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella typhi*, *Klebsiella pneumonia* and *Escherichia coli* by using agar well diffusion method. Colgate-Total brand has the highest mean zone of inhibition (Z.O.I) on the test organisms (30.7 mm) followed by Safi brand (29.2 mm) and Tesco brand (12.5 mm) while Mu'min brand showed the least activity on the test micro-organisms (2.4 mm). The present work found Colgate-total brand toothpaste more effective in controlling pathogenic oral microflora as compared to other brand of toothpaste.

Keywords: Colgate-total, Mu'min, Tesco, Safi, antimicrobial, oral pathogens, zone of inhibition.

1. Introduction

Oral diseases remain a major health problem worldwide [1]. Oral health survey of adults conducted by Ministry of Health Malaysia, Malaysia indicated that the number of periodontal disease is increasing continuously from 1990 to 2010 [2]. There is high correlation between oral diseases and growth of microorganism [3]. Periodontal diseases which is caused by plaque formation is defined as bacterial infections that cause disturbance of the supporting structure of the teeth such as gingival, cementum, periodontal membrane and alveolar bone [4]. Plaque formation results from the accumulation of dietary carbohydrates on the surface of teeth that

25 interact with bacteria. Bacterial metabolic products formed in plaque constantly react with
26 salivary constituents on tooth surface, thus lead to dental caries [5].

27 | There are a number~~s~~s of microorganism~~s~~s which are associated with formation of dental
28 carries such as *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella typhi*, *Klebsiella*
29 *pneumonia* and *Escherichia coli*. Amongst them, *E. coli* is the most common microorganism
30 responsible for the formation of oral cavity [4].

31 Recently, huge numbers of oral hygiene products were introduced to public that claimed
32 to provide maximum protection against periodontal disease. Basically, the efficacy of each
33 toothpaste lies on its capability to retard growth of pathogenic oral microflora. Many oral
34 toothpaste companies also claimed the addition of chemical agents with antiplaque or
35 antimicrobial activity into dental products which served as a potential prophylactic method of
36 reducing plaque mediated disease. This present investigation aimed to investigate antimicrobial
37 efficacy of different commercial toothpastes against selected oral microbiome by using a
38 standard basic procedure. It is noteworthy that, the present result obtained from this research will
39 provide useful evidence to the public to support the benefit of daily usage of current toothpaste to
40 prevent oral disease.

41 2. Materials and Methods

42 2.1 Bacterial strains and growth media

43 The bacteria used in this study were wild type isolates of *Staphylococcus aureus*, *Proteus*
44 *mirabilis*, *Salmonella typhi*, *Klebsiella pneumonia* and *Escherichia coli* which were donated by
45 Mr. Dhana Raj from Asian Institute of Medical Sciences and Technology University (AIMST),
46 Sungai Petani, Malaysia. Microorganisms were maintained using Mueller Hinton Agar (Oxoid,

47 UK)and were sterilized at 121°C and 15 psi for 20 minutes before use. Suspension of each
48 microbial cultures (*S. aureus*, *P. mirabilis*, *S. typhi*, *K. pneumonia* and *E. coli* were prepared
49 accordingly using 0.5 McFarland standards (approx., 10⁸ cfu/mL) for further use in antibacterial
50 assay.

51 2.2 Preparation of toothpaste

52 Toothpastes used in this study were Mu'min premium (Al-Meswak Mu'min, Malaysia),
53 Tesco Pro-tech (Tesco, Malaysia), Safi complete care (Wipro Unza, Malaysia) and Colgate-Total
54 (Colgate Palmolive, Malaysia). All toothpastes were prepared as 2:5 and 4:5 stock concentration
55 by mixing 2 g or 4 g of each various toothpastes in 5 ml of sterile distilled water. As for 100%
56 concentration, toothpastes were directly inserted in the media wells without any dilution.

57 2.3 Antibacterial assay

58 Microorganisms were exposed to the different toothpaste concentrations (2:5 and 4:5)
59 using an agar based assay [6, 7]. 100 µl of each isolates were spread uniformly over Mueller
60 Hinton Agar (MHA) medium by using a cotton swab. Five mm wells were formed on the agar
61 plates using a sterile cork borer and aliquots (100 µL) of each toothpaste each at different
62 concentrations were deposited into the well (Figure 1). The well at the center was placed with tap
63 water which acts as a negative control. Each experiment for different toothpastes was done in
64 triplicate. Inhibition activities against microbial strains were determined by measuring the zones
65 of inhibition formed around the well in millimeter (mm) after 24 h of incubation at 37°C.



66

67 **Figure 1.** Bacterial isolates on MHA agar exposed to different toothpaste of same concentrations

68

69 **2.4 Statistical Analysis**

70 Statistical analyses were performed using GraphPadPrism 5 (GraphPad Software Inc.,
 71 San Diego, CA, EUA) by applying one way ANOVA to assess the significance of change
 72 between experimental groups and control (tap water). The data were expressed as mean \pm
 73 Standard Deviation (SD) and p-value <0.05 was considered as statistically significance.

74

75 **3. Results**

76 The composition on the label of the different toothpaste brands used in this study is shown in
 77 Table 1. All the toothpaste brands contain the same ingredients which were sodium lauryl
 78 sulfate, sorbitol and flavour. Both Colgate-total and Safi brand contain triclosan.

79 **Table 1.** Composition of toothpaste as per stated in the packaging.

Toothpaste	Composition
Colgate-Total	Sodium fluoride, water, triclosan, sorbitol, hydrated silica, sodium lauryl sulfate, flavor, carrageenan, sodium hydroxide, sodium fluoride, sodium saccharin, triclosan
Safi	Dicalcium phosphate dihydrate, water, sorbitol, glycerin, sodium lauryl sulfate, flavour, sodium fluoride, xanthan gum, sodium saccharin, triclosan, tetrasodium pyrophosphate, hydroxyethylcellulose, calcium lactate, piper betle leaf extract, salvadora persica (sugi) bark/root extract.
Tesco	Aqua, sorbitol, hydrated silica, glycerin, sodium lauryl sulfate, aroma, cellulose gum, sodium bicarbonate, zinc citrate, sodium fluoride, sodium saccharin, allantoin, hydroxyethylcellulose, limonene, sodium fluoride.
Mu'min	Calcium carbonate, water, hydrated silica, sorbitol, glycerin, sodium lauryl sulfate, flavor, dicalcium phosphate dihydrate, cellulose gum, mentha piperita (peppermint) leaf extract, calcium phosphate, sodium saccharin, sodium

benzoate, xylitol.

80

81 Table 2 shows the inhibition zone (mm) of the various toothpastes used against the test micro-
82 organisms. The result revealed that Colgate-Total brand has the highest mean zone of inhibition (Z.O.I)
83 on the test organisms (30.7 mm) followed by Safi brand (29.2 mm) and Tesco brand (12.5 mm), while
84 Mu'min brand showed the least activity on the test micro-organisms (2.4 mm). Tap water does not show
85 any antibacterial against any of the tested microbial species (Table 2).

86

87 **Table 2.** Zone of inhibition (mm) of different toothpastes brand.

Toothpaste brands	Test organism	Z.O.I at 2:5 concentration (mm)	Z.O.I at 4:5 concentration (mm)	Z.O.I at 100% concentration (mm)	Average Z.O.I (mm)
Mu'min	<i>S. aureus</i>	17.0 ± 1.4	18.0 ± 1.3	19.0 ± 1.2	12.0
	<i>P. mirabilis</i>	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
	<i>K. pneumoniae</i>	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
	<i>S. typhi</i>	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
	<i>E. coli</i>	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0
Mean					2.4
Safi	<i>S. aureus</i>	42.0 ± 1.4*	40.0 ± 0.0*	44.0 ± 0.0*	42.0
	<i>P. mirabilis</i>	21.0 ± 1.6	31.0 ± 2.1*	30.0 ± 2.2*	27.3
	<i>K. pneumoniae</i>	21.0 ± 1.7	25.0 ± 2.3	28.0 ± 1.8*	24.7
	<i>S. typhi</i>	25.0 ± 1.1	15.0 ± 1.6	31.0 ± 2.0	23.7
	<i>E. coli</i>	27.0 ± 1.2	27.0 ± 1.4	31.0 ± 2.4	28.3
Mean					29.2
Tesco	<i>S. aureus</i>	29.0 ± 1.6*	27.0 ± 1.4*	20.0 ± 1.3	25.3
	<i>P. mirabilis</i>	15.0 ± 1.2	16.0 ± 1.3	16.0 ± 1.2	15.7
	<i>K. pneumoniae</i>	11.0 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	3.7
	<i>S. typhi</i>	15.0 ± 1.2	14.0 ± 1.3	0.0 ± 0.0	9.7
	<i>E. coli</i>	13.0 ± 1.3	11.0 ± 0.6	0.0 ± 0.0	8.0
Mean					12.5
Colgate-Total	<i>S. aureus</i>	40.0 ± 2.1*	42.0 ± 2.4*	44.0 ± 2.0*	42.0
	<i>P. mirabilis</i>	27.0 ± 1.6	28.0 ± 1.4	30.0 ± 2.1	28.3
	<i>K. pneumoniae</i>	24.0 ± 2.0	25.0 ± 1.2	29.0 ± 0.0	26.0
	<i>S. typhi</i>	30.0 ± 1.9*	29.0 ± 1.4*	31.0 ± 1.8*	30.0
	<i>E. coli</i>	27.0 ± 1.7*	26.0 ± 1.6	29.0 ± 1.5*	27.3
Mean					30.7
Tap water	<i>S. aureus</i>			0.0 ± 0.0	0.0
	<i>P. mirabilis</i>			0.0 ± 0.0	0.0
	<i>K. pneumoniae</i>			0.0 ± 0.0	0.0
	<i>S. typhi</i>			0.0 ± 0.0	0.0
	<i>E. coli</i>			0.0 ± 0.0	0.0

88 Z.O.I; zone of inhibition; n=3. *p<0.05

89 Figure 2 depicts that Mu'min brand toothpaste showed antibacterial activity against *S.*
90 *aureus* in dose dependent manner. Mu'min brand toothpaste does not possess any antibacterial
91 [ingredient](#) against other microorganisms tested. Figure 3 showed that Colgate-total brand
92 possessed an antibacterial activity against all the tested microbial strains in dose dependent

93 manner. The most prominent activity of Colgate-total brand was against *S. aureus*. Tesco brand
 94 toothpaste brand showed antimicrobial effect against some of the tested microorganisms. As
 95 depicted in Figure 4, Tesco brand toothpaste brand showed statistically significant antimicrobial
 96 against *S. aureus* at 2:5 (Z.O.I of 29 mm). However, the antibacterial activity against *S. aureus*
 97 was found to be reduced with the increase in toothpaste concentration (4:5; Z.O.I: 27 mm; 100%;
 98 Z.O.I: 20 mm) (Table 2). Figure 3 also indicated that Tesco brand toothpaste showed the highest
 99 Z.O.I at 2:5 as compared to 4.5 mg/ml and 100% against the entire microorganisms tested except
 100 *P. mirabilis*. Tesco brand toothpaste showed maximum zone of inhibition against *P. mirabilis* at
 101 4:5 (16 mm) as compared to 2:5 (15 mm). However, there is no increase in antimicrobial effect
 102 (Z.O.I; 16 mm) of Tesco brand toothpaste against *P. mirabilis* with the increase in concentration
 103 (100%). Safi brand toothpaste also possessed promising antimicrobial activity against the entire
 104 tested microorganism especially *S. aureus* with average zone of inhibition of 29.2 mm (Figure
 105 5).

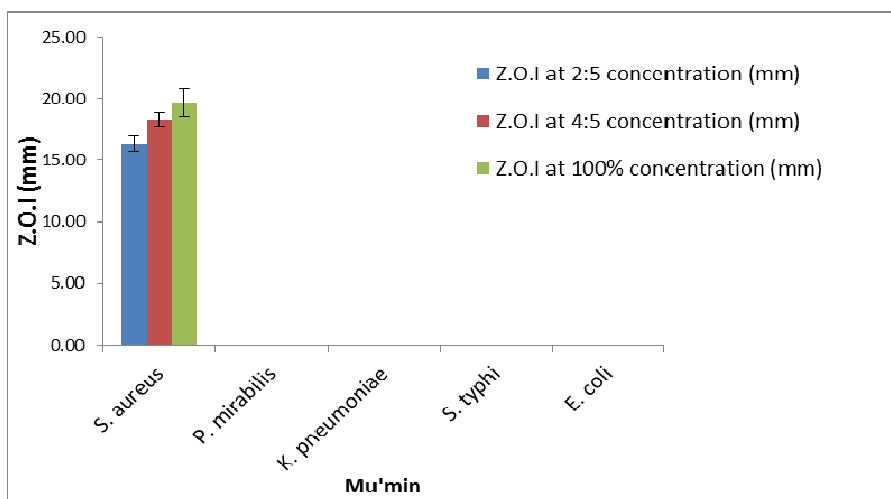


Figure 2. Zone of inhibition of Mu'min brand toothpaste against microorganisms.

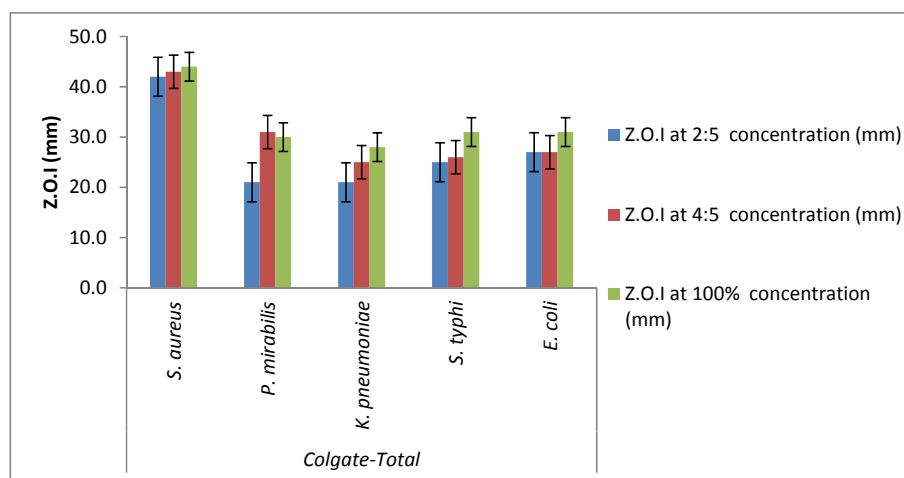


Figure 3. Zone of inhibition of Colgate-Total brand toothpaste against tested microorganisms.

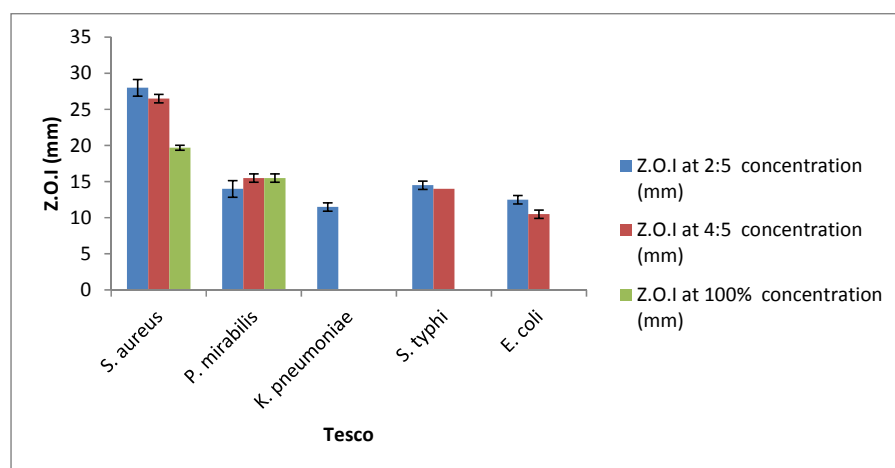
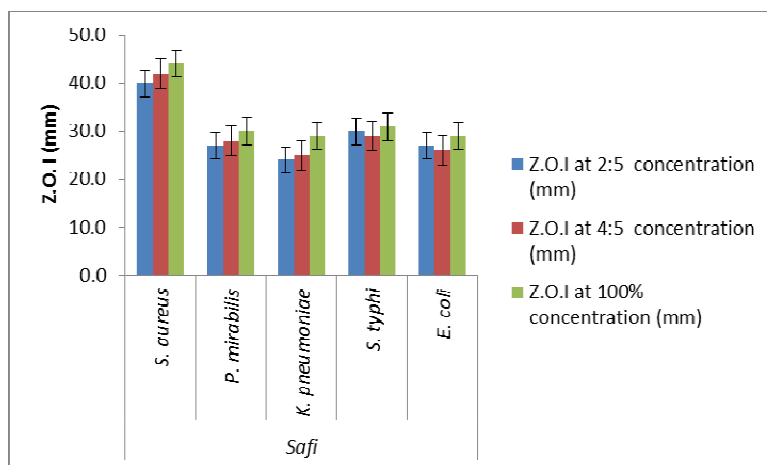


Figure 4. Zone of inhibition of Tesco brand toothpaste against tested microorganisms.



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114 **Figure 5.** Zone of inhibition of Safi brand toothpaste against tested microorganisms.

115 4. Discussion

116 The main step towards a healthy and good looking tooth is maintenance of good oral
 117 hygiene. A good oral hygiene will prevent growth of microbial species that can eventually cause
 118 mouth odour and dental plaque [4]. Hence, there is a massive need to minimize the growth of
 119 microorganisms by using antimicrobial agent [8]. In current scenario, there is huge number of
 120 companies promoting their toothpaste by claiming the presence of special active ingredients
 121 which could protect the teeth against decay. The active ingredients in oral products act by
 122 different mechanism to show their bactericidal effect. It may act via disruption of
 123 microorganism's cell wall, inhibition of microbial enzyme activity or by slowing multiplication
 124 process of microbial species [9]. The well diffusion antimicrobial assay served as an established
 125 method to determine antimicrobial activity of herbs, commercial oral product and cosmetic
 126 product [10].

127 As showed in Figure 3, Colgate-Total brand toothpaste showed highest inhibition zone in
128 average (30.7 mm) as compared to other toothpaste brand against all the tested microbial with
129 most promising activity against *S. aureus*. The presence of fluoride as the active ingredient in
130 this toothpaste might reduce the number of streptococcal colony forming unit that is responsible
131 for dental plaque formation [11]. Safi brand toothpaste also possessed promising antimicrobial
132 activity against the entire tested microorganism with average zone of inhibition of 29.2 mm. The
133 presence of triclosan and sodium fluoride in the Colgate-total brand and Safi toothpaste might
134 contribute to the significant antimicrobial activity of Colgate-total and Safi brand toothpaste
135 (Table 1). It was reported that triclosan and sodium fluoride could reduce 20% of oral bacteria
136 formation [12]. In addition, triclosan has been used over 30 years in oral hygiene product
137 industry for toothpaste and mouth-rinses formulation due to its antibacterial and antifungal effect
138 [13]. In the present study, it was revealed that the presence of piper betle leaf extract and
139 *Salvadora persica* (sugi) bark/root extract might promote the antibacterial effect of Safi brand
140 toothpaste, but it is not equally effective as Colgate-Total brand formulation. However, Safi
141 brand toothpaste showed better antimicrobial effect as compared to Mu'min and Tesco brand
142 toothpaste. This was in accordance with the review data by Moran *et al.* that indicated the
143 efficacy of herbal toothpaste in reducing plaque formation [14]. Mu'min and Tesco brand
144 toothpastes might have least antimicrobial effect against studied microbial strain due to lack of
145 active ingredients present in its toothpaste formulation.

146 Figure 4 indicated that 2:5 mg/mL concentration of Tesco brand toothpaste showed the
147 highest Z.O.I at 2:5 as compared to 4.5 mg/ml and 100% against the entire microorganisms
148 tested except *P. mirabilis*. This result indicated that the antimicrobial activity of this toothpaste
149 against all the tested microorganisms except *P. mirabilis* is at maximum in diluted form (2:5).

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150 As shown in Table 2, Mu'min brand toothpaste showed the least inhibitory effect (mean
151 Z.O.I; 2.4) against all the tested microorganisms as compared to other brand toothpaste. Mu'min
152 brand toothpaste showed dose dependent zone of inhibition (2:5; 17 mm; 4:5; 18 mm and 100%;
153 19 mm). There was no significant zone of inhibition recordedwas obtained for Mu'min brand
154 toothpaste against *P. mirabilis*, *K. pneumoniae*, *S. typhi* and *E. coli*.

155 5. Conclusion

156 The present work has shown that Colgate-total brand toothpaste was more effective in
157 controlling oral microflora as compared to other brand of toothpaste. This result will provide
158 useful evidence to the public to support the benefit of daily usage of current toothpaste to
159 prevent oral disease.

160 Conflict of interest statement

161 We declare that we have no conflict of interest. This research was not funded by any of the
162 toothpaste companies.

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