

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. All the different toothpastes brand was 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 microorganisms (2.4 mm). The present work has found to be that Colgate-total brand toothpaste was 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 as 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 disease and growth of microorganism [3]. The microbial infection may cause destruction of dental tissue leading to periodontal disease [4]. 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

25 bone [3]. Plaque formation results from the accumulation of dietary carbohydrates on the surface
26 of teeth that interact with bacteria. Bacterial metabolic products formed in plaque constantly
27 react with salivary constituents on tooth surface, thus lead to dental caries [5].

28 There are numbers of microorganism which are associated with formation of dental
29 carries such as *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella typhi*, *Klebsiella*
30 *pneumonia* and *Escherichia coli*. Amongst them; *E. coli* is the most common microorganism
31 responsible for the formation of dental caries due to oral cavity [4].

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

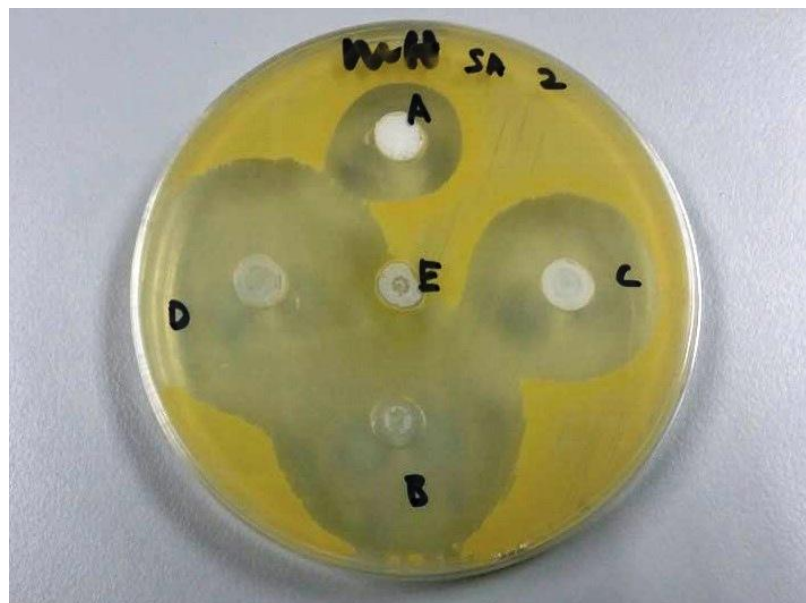
47 UK)and were sterilized at 121°C and 15 psi for 20 minutes before use. Suspension of each
48 microbial isolates (*S. aureus*, *P. mirabilis*, *S. typhi*, *K. pneumonia* and *E. coli* were prepared
49 accordingly using 0.5 McFarland standards (approx., 10^8 cfu/mL) for further use in antibacterial
50 assay.

51 **2.2 Preparation of toothpaste**

52 Toothpaste 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 concentration (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 5mm wells was formed on the agar
61 plates using a sterile cork borer and aliquots 100 µL of each toothpaste at different concentration
62 were deposited into the well (Figure 1). The well at the center was placed with tap water which
63 acts as a control. Each experiment for different toothpastes was done in triplicate. Inhibition
64 activities against microbial strains were determined by measuring the zones of inhibition formed
65 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
85 showed any antibacterial against any of the tested microbial species (Table 2).

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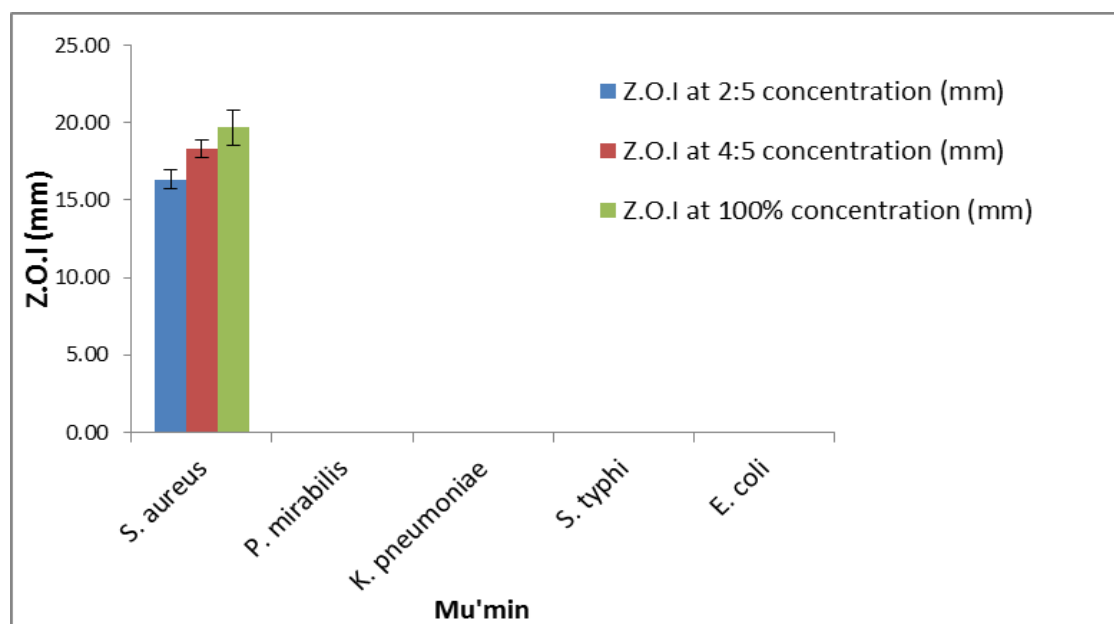
87 **Table 2.** Zone of inhibition (mm) of different toothpastes brand.

| 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 activity against other microorganisms tested. Figure 3 showed that Colgate-total brand possessed an
92 antibacterial activity against all the tested microbial strains in dose dependent manner. The most

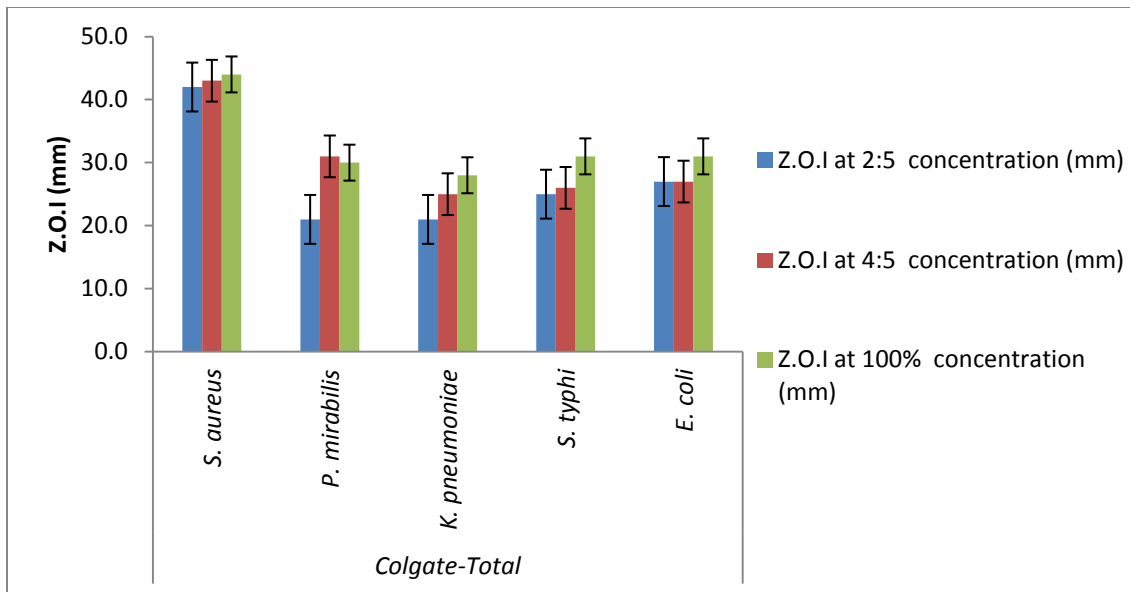
93 prominent activity of Colgate-total brand was against *S. aureus*. Tesco brand toothpaste brand
 94 showed antimicrobial effect against some of the tested microorganisms. As depicted in Figure 4,
 95 Tesco brand toothpaste brand showed statistically significant antimicrobial against *S. aureus* at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



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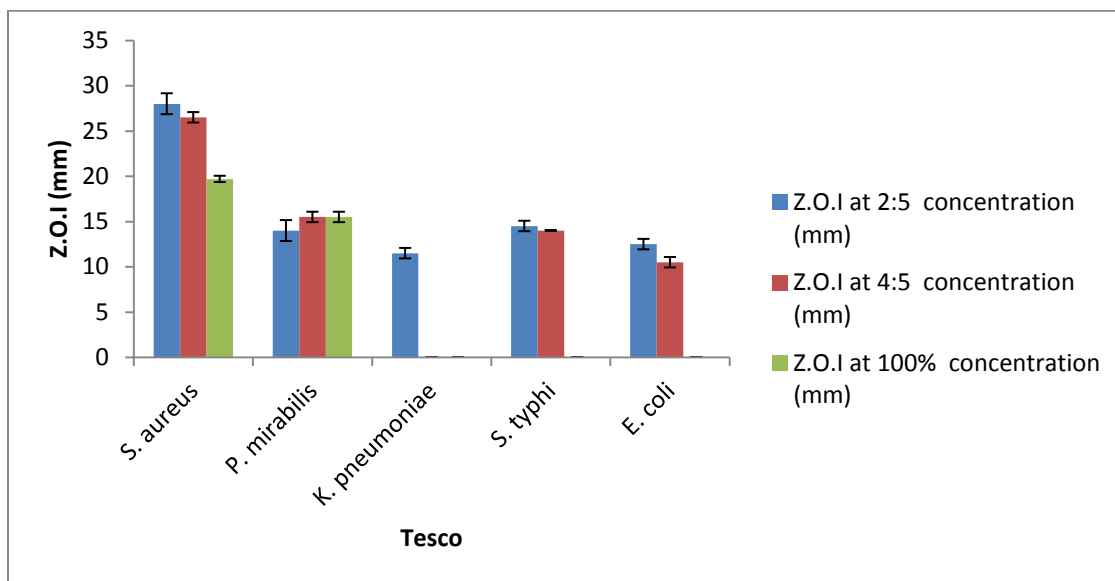
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Figure 2. Zone of inhibition of Mu'min brand toothpaste against microorganisms.



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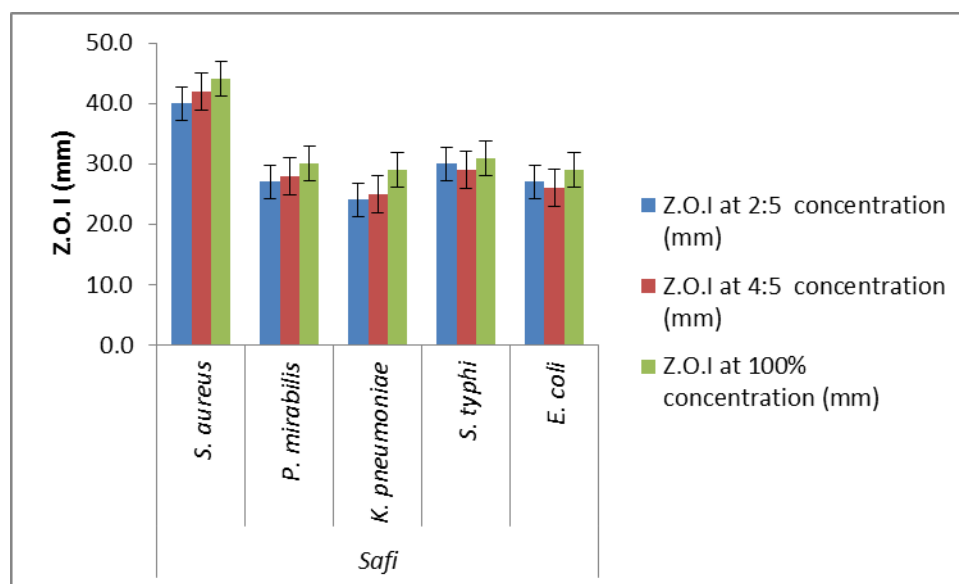
108 **Figure 3.** Zone of inhibition of Colgate-Total brand toothpaste against tested microorganisms.



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

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

114 4. Discussion

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

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

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

147 As shown in Table 2, Mu'min brand toothpaste showed the least inhibitory effect (mean
148 Z.O.I; 2.4) against all the tested microorganisms as compared to other brand toothpaste. Mu'min

149 brand toothpaste showed dose dependent zone of inhibition (2:5; 17 mm; 4:5; 18 mm and 100%;
150 19 mm). There was no significant zone of inhibition was obtained for Mu'min brand toothpaste
151 against *P. mirabilis*, *K. pneumoniae*, *S. typhi* and *E. coli*.

152 **5. Conclusion**

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

157 **Conflict of interest statement**

158 We declare that we have no conflict of interest.

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