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Research paper

An *in Vitro* Analysis of Antibacterial Efficacy of Local Brands of Toothpastes and Mouthrinses in Malaysian Market on Selected Oral Pathogens

M Chandramohan*, S W Chan[1], P Paulraj, P T Mohamed Javad, P Sajeesh, K P Sajna, T Ketharin

Department of Biomedical Sciences, Faculty of Medicine, MAHSA University, Bandar Saujana Putra, Selangor, Malaysia
**Corresponding author E-mail: chandramohan@mahsa.edu.my*

Abstract

Oral disease affects a considerable portion of population and is considered one of the major causes of tooth loss in developed and developing countries. An *in vitro* study was conducted to investigate the antimicrobial efficacy of toothpastes and mouthrinses towards oral pathogens which are found to cause most of the oral diseases such as gingivitis and dental plague. In this study, a total of five toothpastes and four mouthrinses were investigated for their antimicrobial activity against five oral pathogens such as *Streptococcus salivarius*, *Streptococcus sanguis, Streprococcus oralis, Streptococcus mutans* and *Candida albicans.* The efficacy of different concentration of the toothpastes and mouthrinses were assessed by agar well diffusion method. Statistical analysis was performed by using analysis of variance (ANOVA) with post-hoc least square differences (LSD) method (p=0.05). Toothpaste B gave the maximum zone of inhibition against tested organisms, *Streptococcus sanguis* and *Streptococcus oralis*. Toothpaste C and E gave the maximum zone of inhibition against *Streptococcus salivarius* and *Streptococcus mutans* respectively. Toothpaste A was most effective against *Candida albicans*. Mouthrinse G was most effective against *Streptococcus salivarius, Streptococcus sanguis, Streptococcus oralis* and *Candida albicans.* In conclusion, the present study has demonstrated that dentifrices which contain fluoride and cetylpyridium chloride formulation gave the maximum zone of inhibition against the tested organisms compared to other active ingredients.

Keywords: *Antimicrobial efficacy; Cetylpyridium chloride; Fluoride; Mouthrinse; Toothpaste.*

1. Introduction

Mouth is the major gateway to the body, whatever affects oral health may also affects general health [1]. Therefore, oral health is important for maintaining the health of orofacial complex which include teeth, gums and tissues of face [2]. Oral health has been linked with systemic infections, autoimmune disorders, glucose control, and other diseases **[**3]. There are some normal flora found in mouth that are important for prevention of colonization by pathogenic microbes and maintaining the health of oral cavity [4-5]. Tooth surface breakdown and subsequent cavity formation can be caused by organic acids, which are produced as the by-products of fermentation, dissolve the hydroxyapatic component of enamel and dentine [6]. The bacteria will form coating film on teeth, mucous membrane, dorsum of tongue [7] which is known as dental plague and it will cause harmful activities if it is not cleaned properly **[**8]. Certain studies showed that oral cavity is an ideal environment for microorganism to grow due to the moist and warm environment and special anatomic structure present in oral cavity [9]. Oral problems occur commonly among population in developed or developing countries and affects people from all ages of life. Several measures were offered for maintaining good oral hygiene and to prevent oral diseases. However, even most of the children's around age ten have basic knowledge of dental health such as importance of proper brushing, they still fail to brush their teeth to clean and tend to consume cariogenic foods, underestimate health risks and tend to oppose their parents and teachers, making it the most difficult period for health education [10]. Food debris is the driving force of cavity formation and subsequently teeth are vulnerable to dental plaque which allows bacteria to attack the enamel [11]. On the other hand, dental caries is also classified as tooth decay where microorganism will ferment dietary carbohydrates to form lactic acids or others type of acid. These types of acids might cause localized destruction and demineralization towards teeth [12]. The incidents of oral problem still occur mainly due to eating habit by increased consumption of high sugar food [13]. In oral cavity, previous studies showed that several types of microorganism are associated with dental caries which include *Streptococcus salivarius, Streptococcus sanguis, Streptococcus oralis, Streptococcus mutans* or *Candida albicans*. *Streptococcus salivarius, Streptococcus sanguis* and *Streptococcus oralis* which are known to directly bind and colonize the teeth surface to form dental plaque, oral caries and periodontal disease [14-15]. *Streptococcus mutants* ferment the carbohydrates and cause production of acids which lead to demineralization of teeth. Reports suggest that *Candida albicans* is also able to cause active caries lesion [16].

There are different chemical and mechanical methods offered for maintaining good oral hygiene and to overcome problems of oral disease. The mechanical method is brushing teeth prevent the plaque to remain on surface of teeth for a longer period [17]. The purpose is to clean and remove the presence of food debris, stains and prevent tooth surface from dental plaque [18]. It is now proved that tooth brushing alone only can remove 50% of dental

plaque and the additional mechanical and antimicrobial measures such as use of mouthwash or flossing teeth with dental floss are required to further reduce bacterial load [19]. Toothpaste is a useful semi-solid dentifrice that can improve health of oral cavity and teeth, eliminate halitosis, dental plaque and food residue [20]. Non herbal toothpastes usually contain some active ingredients such as fluoride, sodium monofluorophosphate, triclosan or xylitol [21]. However, herbal toothpastes consist of herbal or plant extract, neem oil or sages which are able to inhibit oral pathogens in oral cavity [22]. On the other hand, mouthrinse is a liquid dentifrice which is used to reduce oral pathogen and acts as saliva substitutes to neutralize acid and avoid xerostomia [23]. Cosmetic mouthrinse may control bad breath temporary and leave a pleasant odour on mouth but it does not contains any chemical application beyond their temporary benefit whereas for therapeutic mouthrinse, it contains active ingredients to help in reduce dental diseases. There are several studies which claim that active ingredients found in toothpastes and mouthrinses are able to control the plaque causing microbes, but very little studies have been made to investigate these claims.

2. Materials and Methods

2.1 Selection of toothpastes and mouthrinses

Some of the commercially available toothpastes and mouthrinses in Malaysian market were subjected for the study which includes, Darlie Fluoride toothpaste, Oral B toothpaste, Amboinense toothpaste, Himalaya Herbal toothpaste, Aeon Big Value Sensitive toothpaste, Listerine Total Care mouthrinse, Oral B Pro Health mouthrinse, Betadine gargle and mouthwash and Thymol mouthwash (Table 1).

2.2 Test organisms

The test organisms used for the current study were the pure culture of the pathogens procured from American Type Culture Collection (ATCC) which includes *Streptococcus salivarius* (ATCC 13419), *Streptococcus sanguis* (ATCC 10556), *Streptococcus oralis* (ATCC 6249), *Streptococcus mutans* (ATCC 2175), *Candida albicans* (ATCC 332767).

2.3 Evaluation of dentifrices

The selected dentifrices solutions were made by mixing 3 grams of each toothpaste in 3ml of pyrogen free distilled water to give 1:1 dilution; the dentifrices were further diluted in pyrogen free distilled water to produce 1:2, 1:4, 1:8 and 1:16 dilutions. Similarly, 3ml of mouthrinse with 3ml of pyrogen free distilled water was used to give 1:1 dilution and serial dilution were made as above [16].

2.4 Antimicrobial assay

Mueller Hinton agar was used to assess the antimicrobial activity. Five oral pathogens were swabbed onto Mueller Hinton agars by sterile cotton swab. The agars were then allowed to dry for around 15 to 20 minutes. Sterile cork borer was used to punch five wells at equidistance in each of plates. 200µl of dentifrices dilutions were pipetted into each of the five wells according to the different dilution. For control, distilled water was pipetted onto Mueller Hinton agar with punched well. After the dentifrices were added into each well, the plates were incubated for 24 hours at 37^0C. All the plates were made in triplicates and the assay was repeated thrice [24].

2.5 Statistical analysis

The zones of inhibition were calculated by the average of vertically and horizontally measured diameter of the zone of inhibition. Statistical analysis was performed by using statistical package SPSS windows version 22 by applying mean values using analysis of variance (ANOVA) with post-hoc least square differences (LSD) method (p=0.05) [24].

Table 1: Selected dentifrices

No.	Toothpastes	Mouthrinses
1.	Darlie Fluoride (A)	Listerine Total Care (F)
2.	Oral-B (B)	Oral B Pro Health (G)
3.	Aeon Big Value Sensitive (C)	Betadine Gargle and Mouthwash (H)
4.	Himalaya Herbal (D)	Thymol (I)
5.	Amboinense (E)	

3. Results and discussions

3.1 Antimicrobial efficacy study

The maintenance of oral health is the important key to prevent the growth of dental diseases. Frequent consumption of high sugar food will develop oral diseases [25]. Results of the present study (Table 2) in comparison of *S. salivarius* among the different toothpastes and mouthrinses at a concentration of 1:1, 1:2, 1:4, 1:8 and 1:16 showed that toothpaste C (Aeon Big Value Sensitivity) had the maximum zone of inhibition which was extremely significant ($p<0.001$) while toothpaste D (Himalaya) gave the minimum zone of inhibition which was also extremely significant ($p<0.001$). Among the different mouthrinses studied mouthrinse G (Oral B) gave the maximum zone of inhibition which was statistically significant ($p<0.05$) while mouthrinse F (Listerine) gave the minimum zone of inhibition ($p<0.01$).

Against *S. sanguis*, among the different toothpastes and mouthrinses in concentration of 1:1, 1:2, 1:4, 1:8 and 1:16 (Table 3) toothpaste B (Oral B) gave the maximum zone of inhibition which was extremely significant ($p<0.001$) while toothpaste A (Darlie) gave the minimum zone of inhibition ($p<0.001$). In case of mouthrinses the mouthrinse G (Oral B) gave the maximum zone of inhibition which was statistically significant ($p<0.05$) while mouthrinse F gave the minimum zone of inhibition which was extremely significant ($p<0.001$). Similarly, in comparison of *S. oralis* among the different toothpastes and mouthrinses in concentration of 1:1, 1:2, 1:4, 1:8 and 1:16 (Table 4) showed that toothpaste B gave the maximum zone of inhibition ($p<0.001$) while toothpaste D gave the minimum zone of inhibition which was statistically significant ($p<0.05$). Result of mouthrinses showed that mouthrinse G gave the maximum zone of inhibition which was extremely significant ($p<0.001$) while mouthrinse F and I (Thymol) gave no zone of inhibition.

Results showed (Table 5) that toothpaste E (Amboinense) gave the maximum zone of inhibition against *S. mutans* which was extremely significant ($p<0.001$) while toothpaste D gave the minimum zone of inhibition ($p<0.01$). Result also showed that mouthrinse F gave the maximum zone of inhibition while mouthrinse G gave the minimum zone of inhibition which was extremely significant ($p<0.001$). However, in comparison of *C. albicans* among the different toothpastes and mouth-rinses in concentration of 1:1, 1:2, 1:4, 1:8 and 1:16 (Table 6) showed that toothpaste A gave the maximum zone of inhibition which was extremely significant ($p<0.001$) while toothpaste C gave the minimum zone of inhibition ($p<0.001$). Among the mouthrinses, the mouthrinse G gave the maximum zone of inhibition which was very significant ($p<0.01$) while mouth-rinse F gave no zone of inhibition.

Table 2: Antimicrobial activity of dentifrices formulations against *Streptococcus salivarius*.

Dentifrices		Zone of Inhibition (mm)				
		1:1 Dilution Mean ± Standard Deviation	1:2 Dilution Mean ± Standard Deviation	1:4 Dilution Mean ± Standard Deviation	1:8 Dilution Mean ± Standard Deviation	1:16 Dilution Mean ± Standard Deviation
Control	Distilled Water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Non Herbal Toothpastes	A	22.67±2.52*	21.00±4.00*	19.83±3.25*	17.50±3.12*	12.50±3.77*
	B	24.50±0.87***	23.17±1.26***	18.50±1.50***	16.17±0.76***	12.00±2.65***
	C	30.00±1.00***	25.17±0.76***	22.00±2.00***	20.33±2.52***	14.33±2.50***
Herbal Toothpastes	D	20.50±0.50***	18.83±1.26***	17.17±1.90***	11.50±0.50***	11.00±1.00***
	E	23.00±2.00*	20.83±3.88*	20.33±3.79*	16.67±2.89*	12.33±3.06*
Mouth rinses	F	9.33±0.58**	8.50±0.00**	8.17±0.29**	8.17±0.29**	8.17±0.29**
	G	18.33±0.58*	15.17±2.93*	14.33±2.52*	13.17±2.02*	10.67±2.31*
	H	12.17±1.89**	9.50±0.50**	8.67±0.29**	8.50±0.00**	8.17±0.29**
	I	9.50±0.50**	8.33±0.29**	8.33±0.29**	8.17±0.29**	8.17±0.29**

n=3, * statistically significant p<0.05, **very significant p<0.01, ***extremely significant p<0.001

Table 3: Antimicrobial activity of dentifrices formulations against *Streptococcus sanguis*.

Dentifrices		Zone of Inhibition (mm)				
		1:1 Dilution Mean ± Standard Deviation	1:2 Dilution Mean ± Standard Deviation	1:4 Dilution Mean ± Standard Deviation	1:8 Dilution Mean ± Standard Deviation	1:16 Dilution Mean ± Standard Deviation
Control	Distilled Water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Non Herbal Toothpastes	A	20.00±2.00***	17.00±1.00***	15.50±0.50***	14.33±0.58***	11.00±1.00***
	B	24.00±1.50***	17.67±0.58***	16.50±0.50***	13.83±0.29***	11.17±1.04***
	C	20.67±1.53**	18.33±2.87**	17.00±2.00**	14.33±0.58**	10.50±3.12**
Herbal Toothpastes	D	22.33±1.15***	18.33±2.89***	14.83±2.57***	12.67±2.89***	9.33±1.15***
	E	21.00±2.65***	18.33±1.53***	13.67±4.04***	9.83±1.04***	8.17±0.29***
Mouth rinses	F	9.67±0.58***	8.50±0.50***	0.00±0.00	0.00±0.00	0.00±0.00
	G	22.33±6.0*	16.33±2.3*	15.00±2.6*	12.67±1.5*	12.00±1.0*
	H	11.00±1.0***	9.00±0.50***	8.67±0.58***	8.17±0.29***	0.00±0.00
	I	13.17±1.61***	12.00±0.00***	11.17±0.29***	10.17±0.76***	8.50±0.50***

n=3, * statistically significant p<0.05, **very significant p<0.01, ***extremely significant p<0.001

The data of the present study reveals clearly that all the investigated toothpastes and mouthrinses produced wide variations in their effectiveness against five tested oral pathogens. Among all the investigated toothpaste, toothpaste B was considered most effective against two oral pathogens, based on the mean diameter of zone of microbial inhibition in agar well diffusion method. Toothpaste B gave the highest zone of inhibition against two microorganisms, *S. sanguis* and *S. oralis* compared to other brand of dentifrices. However, toothpaste C and toothpaste A were effective against *S. salivarius* and *C. albicans* respectively due to they gave the maximum zone of inhibition. These toothpastes fall under group of non-herbal toothpaste and all of them are found to be effective against four of the target microorganisms. This might be due to presence of fluoride as active ingredients in these formulations. Fluoride is a chemical compound which used to control tooth decay and oral caries. Fluoride is also mentioned as another form in oral care products which is sodium monofluorophosphate (MNF). It is commonly used in most of the oral care products in order to help in prevention of dental caries. Toothpaste A, B and C consists of fluoride and sodium monofluorophosphate as the active ingredients where they are able to eliminate most of the *Streptococcus* strains. However, if the amount of bacteria is too high or the toothpaste was diluted in 1:16 dilution, it is difficult to eliminate the bacteria completely [26]. Studies conducted by Kay and Locker (1998), Sheiham (2001), Eaton and Carlile (2008), and Santos *et al.,* (2013) mentioned that regular brushing with fluoride toothpaste is effective to reduce dental caries [27-30]. Similar studies conducted by Cenci *et al.,* (2008) proved that fluoride concentration in saliva will increase dramatically when brushing with fluoride toothpaste. Once the fluoride concentration become high in dental biofilm, it can be maintained at higher amount for a longer period [31]. Thus, it is not only to enhance dental remineralization but also minimize the demineralization on surface covered by biofilm remnants [32-33]. Fluoride exhibits antibacterial and antifungal effects such as prevention of dental plaque and metabolic interference [34]. Adwan *et al.,* (2012) studies demonstrated that sodium monofluorophosphate have antimicrobial effects against *C albicans* but the effects are less and needs to be combined with other active ingredients. Toothpaste which combined sodium fluoride with herbal extract showed a better antimicrobial action against *C albicans* compared to toothpaste with fluoride only [35].

Table 4: Antimicrobial activity of dentifrices formulations against *Streptococcus oralis*.

Dentifrices		Zone of Inhibition (mm)				
		1:1 Dilution Mean ± Standard Deviation	1:2 Dilution Mean ± Standard Deviation	1:4 Dilution Mean ± Standard Deviation	1:8 Dilution Mean ± Standard Deviation	1:16 Dilution Mean ± Standard Deviation
Control	Distilled Water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Non Herbal Toothpastes	A	23.67±1.53*	21.00±2.65*	19.67±2.25*	18.50±2.29*	15.50±1.80*
	B	27.00±2.65***	25.33±3.51***	17.17±3.69***	14.83±1.61***	9.67±1.53***
	C	26.33±6.81*	21.50±0.87*	17.83±2.02*	14.17±3.89*	12.67±5.03*
Herbal Toothpastes	D	22.67±4.04*	19.67±3.40*	18.67±3.75*	15.33±1.53*	12.33±4.04*
	E	24.67±4.51*	18.67±3.06*	16.00±3.46*	13.83±4.01*	9.67±1.53*
Mouth rinses	F	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	G	23.50±3.04***	14.83±4.48***	11.00±1.00***	10.50±0.87***	8.67±1.15***
	H	10.33±1.53***	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	I	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

n=3, * statistically significant p<0.05, **very significant p<0.01, ***extremely significant p<0.001

Table 5: Antimicrobial activity of dentifrices formulations against *Streptococcus mutans*.

Dentifrices		Zone of Inhibition (mm)				
		1:1 Dilution Mean ± Standard Deviation	1:2 Dilution Mean ± Standard Deviation	1:4 Dilution Mean ± Standard Deviation	1:8 Dilution Mean ± Standard Deviation	1:16 Dilution Mean ± Standard Deviation
Control	Distilled Water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Non Herbal Toothpastes	A	22.50±1.50**	20.00±1.00**	19.17±1.61**	17.17±2.02**	16.00±1.32**
	B	21.33±0.76***	18.67±0.58***	14.67±1.53***	12.33±2.52***	8.33±0.58***
	C	22.00±1.80***	20.83±3.25***	18.33±1.76***	15.67±1.53***	10.83±1.26***
Herbal Toothpastes	D	20.00±6.26**	13.67±4.73**	11.00±3.61**	9.67±2.89**	0.00±0.00
	E	25.67±2.52***	23.67±1.53***	21.83±1.89***	16.33±4.04***	9.00±1.73***
Mouth rinses	F	19.00±3.61***	15.33±2.52***	9.50±1.32***	8.33±0.58***	0.00±0.00
	G	12.67±3.51***	9.17±1.04***	0.00±0.00	0.00±0.00	0.00±0.00
	H	14.83±4.3***	10.67±2.08***	9.50±1.50***	8.50±0.87***	0.00±0.00
	I	13.33±2.08***	10.33±1.76***	9.33±1.53***	8.67±1.15***	0.00±0.00

n=3, * statistically significant p<0.05, **very significant p<0.01, ***extremely significant p<0.001

Table 6: Antimicrobial activity of dentifrices formulations against *Candida albicans*.

Dentifrices		Zone of Inhibition (mm)				
		1:1 Dilution Mean ± Standard Deviation	1:2 Dilution Mean ± Standard Deviation	1:4 Dilution Mean ± Standard Deviation	1:8 Dilution Mean ± Standard Deviation	1:16 Dilution Mean ± Standard Deviation
Control	Distilled Water	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Non Herbal Toothpastes	A	26.50±3.28***	24.83±4.25***	22.00±2.65***	19.00±1.32***	11.00±1.00***
	B	21.67±1.15***	18.17±2.36***	14.67±3.62***	10.17±0.76***	8.67±0.29***
	C	19.83±2.57***	16.33±2.08***	13.83±1.61***	12.33±0.76***	9.50±0.50***
Herbal Toothpastes	D	23.33±4.73**	18.00±2.65**	16.00±2.65**	12.50±1.80**	10.33±0.58**
	E	19.83±0.76***	18.33±0.58***	17.67±0.58***	15.00±1.00***	10.67±1.53***
Mouth rinses	F	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	G	22.67±2.52**	15.33±3.79**	12.67±2.52**	11.33±1.53**	9.67±1.53**
	H	16.00±2.00***	9.50±0.50***	8.17±0.29***	0.00±0.00	0.00±0.00
	I	10.00±1.00***	9.50±0.50***	8.67±0.58***	8.17±0.29***	0.00±0.00

n=3, * statistically significant p<0.05, **very significant p<0.01, ***extremely significant p<0.001.

Toothpaste D and E used in our study are herbal toothpaste and the result in present study showed they were less effective compare to other toothpastes. This may due to the presence of herbal extract as active ingredients in these two toothpastes. Using herbal toothpastes as oral health products have become a popular trend among population. Previous *in vitro* studies have proved that herbal toothpastes have antimicrobial actions towards oral pathogens but it does not mentioned that there is any adverse effect seen in herbal toothpaste [36]. From the present study, it showed toothpaste D and E contain *Salvadora persica* (miswak) extract which is a cheap and traditional product present in chewing stick that is important in maintenance of oral health [37]. Olsson and Char *et al.*, studies stated that using oral care products containing *Salvadora persica* extract can effectively control gingivitis and dental plague [38] Olsson study also strongly advice the use of miswak chewing stick due to its affordability [39]. Danielsen *et al.*, studies mentioned combination of toothpaste with miswak chewing stick have more effective action in reducing gingivitis and dental plaque compared to brushing with toothpaste only [40]. Single blind, randomized and crossover study by Almas investigated the antimicrobial effects of *Salvadora persica* extract onto *S. mutans* and the study's result showed there is significant decline in *S. mutans* amount in miswak user [41]. Moreover, few studies were performed to investigate the antimicrobial actions of miswak extract against *Candida albicans*. Al-Bagieh *et al.*, studies mentioned that 15% concentration of *Salvadora persica* extract were effective to against *Candida albicans* [42]. However, another study showed that there is no inhibitory effects against *Candida albicans* was observed even at 50% concentration of *Salvadora persica* extract [43]. Thus, the results on the herbal extract possessing antibacterial or antifungal activity is inconsistent. Hence, herbal product containing toothpastes are not very effective in reducing the oral pathogens according to our study.

With respect to mouthrinse, mouthrinse G has more effective to eliminate *Streptococcus strain* and *C. albicans*. This might be due to the presence of cetylpyridium chloride (CPC). CPC is a cation compound which acts as active ingredients in mouthwash, throat sprays and nasal sprays. The CPC is used widely against dental plaque and gingivitis [44]. Furthermore, Mankodi *et al.*, (2005) studies also recommended that study's subject with mild gingivitis inflammation proved that CPC will be more effective compared to placebo in order to decrease the incidents of dental plaque and gingival bleeding [45]. Besides that, CPC also have antifungal properties towards *C. albicans* [46-47]. Several studies showed that CPC can reduce the amount of fungus and prevent formation of dental plaque [48-49]. The standard concentration of CPC found in common mouthrinse is 0.05%. However, these concentrations will be rapidly diluted in the mouth by saliva and resulted in reduced effectiveness that could lead to the development of fungal resistance [50]. Due to this reason, mouthwashes need to be used several times a day [51].

Mouthrinse F, H and I were less effective as compared to mouthrinse I which might be due to the presence of thymol, 1% w/v of povidone-iodine and menthol as ingredients. Thymol exhibit antibacterial and antifungal actions while menthol is the organic compound from peppermint oil or corn mint. Both of the formulations are used as mouthwash to remove bad mouth odor, relieve minor sore throat and reduce mouth inflammation. Previous studies proved that the mouthrinses which contain thymol and menthol as ingredients have less effectiveness against *C. albicans* and other bacterial strains [52]. Besides that, Neeraja *et al.*, (2008) study stated that povidone-iodine formulation has reduced 28.4% of *S. mutans* count. However, the count started to increase gradually after few hours. It proved that povidone-iodine formulation only have an immediate effect against *S. mutans* [53]. Thus, thymol, povidone-iodine and menthol ingredients in mouthrinses have limited antimicrobial efficacy compared to cetylpyridium chloride formulation mouthrinse [54].

4. Conclusions

In conclusion, the result of this present study showed that toothpaste containing fluoride formulation are more effective compared to herbal extract toothpaste and mouthrinse containing cetylpyridium chloride is more effective than thymol and menthol formulations against the target microorganisms. Although the above study clearly indicates that the tested toothpastes and mouthrinses possess antimicrobial properties, the results need to be analyzed without bias, since the present study is an *in-vitro* study and the efficacy will not be same at *in-vivo* conditions. Oral cavity is a complex environment that is characterized by temperature variations in various parts of the mouth, the presence of saliva, and other factors will reduce the antimicrobial efficacy of the dentifrices tested. Hence, further investigations are needed to confirm the antibacterial efficacy of the dentifrices tested.

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