



**IN VITRO ANTIMICROBIAL EFFICACY OF SOME COMMERCIAL DENTIFRICES
AVAILABLE IN MARKET**

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ABSTRACT

In the present study antimicrobial activity of commercial available dentifrices six chemical synthetic such as Colgate Strong Teeth, Pepsodent, Close up, Colgate gel, Cibaca and Anchor, three herbal such as Babool, Dabur red, Meswak, and seven mouth rinse formulation namely Listerine, Hexidine, Triguard, Senquel, Wokadine, Tantum, Betadine and Sflo were tested against some microorganism such as *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Candida albicans* using a disk diffusion method and Chlorhexidin was used as standard. In synthetic tooth paste Colgate, Pepsodent, Anchor and Cibaca shows potent antibacterial activity. The antibacterial activity among the herbal dentifrices namely Meswak was found the maximum antibacterial activity i.e. 42 mm zone of inhibition at 70% concentration against *Staphylococcus aureus* than other two herbal products i.e. Babool and Dabur red. While Mouth washes Hexidine shows larger zone of inhibition i.e. 15 mm against *Streptococcus mutans*. It was concluded that the synthetic tooth pastes commonly used contain chemical agents, which are known to produce harmful side effects on prolong use. This study suggest that use of herbal tooth pastes which contain extract of medicinal plants and possess a good antimicrobial activity .

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INTRODUCTION

It is generally believed that oral cavity is always at a risk of infection with bacterial pathogens associated with oral cavity. *Streptococcus* constitutes 60 % to 90% of the bacteria that colonize the teeth in the first 4h after professional cleaning (de Rysky, 1988). Other early colonizers include *Actinomyces* spp., *Capnocytophage* spp., *Eikenella* spp., *Haemophilus* spp., *Prevotella* spp., *Propionibacterium* spp., and *Veillonella* spp., many of the physical interaction that occurs between the organisms of this community are known. The ability to bind to other early colonizers and to host molecules may confer an advantage on these viridians streptococci in establishing early dental plaque (Gunsolley, 2006)

Effective antimicrobial agents against these oral pathogens could play an important part in the prevention of dental caries and periodontal diseases particularly those that affect plaque formation. Periodontal diseases are the disease caused with in oral cavity by large number of microorganisms that colonize on the tooth surface and forms dental plaque in sub- gingivalis margin. Colonization of these microorganisms results in the formation of dental biofilm (Bowden, 1976; Bagg, 1999)A wide range of human infection are caused by dental biofilm like dental carries , periodontal ,otitis media, musculoskeletal infection ,necrotizing fasciitis, biliary track infection.

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Characteristics of these infections are the persistence and chronicity of the infections as well as the difficulty in their eradication (Sheen and Moran, 2001). It has been found that 65% of the human infections that infect human being are caused by the microorganisms living inside the biofilm. (Tanner, 1998) Dental plaque is the community of microorganisms found on a tooth surface as a biofilm, embedded in a matrix of polymers of host and bacterial origin (Marsh, 1999).

Antibacterial agents can be divided into two categories according to their chemical composition of the antimicrobial agent, synthetic organic /inorganic agents or herbal agent. The plant (oils) materials with antibacterial activity have long been the goal of medical science from the ancient time. Moreover, it was found that some oils from plants show effective antibacterial activity (Willershausen, 1991).

Consumers of these natural health care products typically are well-educated and have above-median incomes. However, they tend to conceal their outside the- mainstream practices from their health care professionals to avoid ridicule (Marsh, 2000). As the popularity of natural medicines and dentifrices continues to rise, dental professionals are in a position to provide information to patients about these products' safety and efficacy (Mullally, 1995; Settembrini, 1998). A lack of scientific studies on chemical and herbal products in the peer-reviewed dental literature poses a conundrum for health care professionals when dealing with these products. Therefore, the

objectives of the study were to determine an in vitro study to assess the antimicrobial potential of chemical and herbal dentifrices using a standard diffusion method.

MATERIALS AND METHODS

Collection of Sample

Total 14 natural herbal dentifrices (one powder and 13 pastes) were purchased from retail health food stores in Southern California (Table 1). Colgate Total (Colgate-Palmolive, New York City) served as the positive control, and we used sterile pyrogen-free water (Milli-Q system, filtered with Millipak Gamma Gold Filters, Sterile Vented Filter Unit 0.22 µm, Millipore, Bedford, Mass.) as the negative control.

Bacterial cultures: The standard pathogenic bacterial cultures were procured from IMTECH, Chandigarh, India and used in the present study (Table 1). The bacterial cultures were rejuvenated in Mueller- Hinton broth (Hi-media laboratories, Mumbai, India) at 37°C for 18h and then stocked at 4°C in Mueller-Hinton Agar. The inoculum size of the bacterial culture was standardized according to the National committee for Clinical Laboratory Standards (NCCLS, 2002) guideline. The pathogenic bacterial culture was inoculated into sterile Nutrient broth and incubated at 37°C for 3h until the culture attained a turbidity of 0.5 McFarland units. The final inoculum size was standardized to 10⁵ CFU/mL with the help of SPC and Nephlo-turbidometer.

Table 1 Bacterial cultures used in study (IMTECH, Chandigarh, India)

Bacterial Pathogens	MTCC Number
Staphylococcus aureus	96
Streptococcus mutans	497
Lactobacillus acidophilus	10307
Candida albicans	18804

Staphylococcus aureus, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Candida albicans* were procured from Institute of Microbial Technology (IMTECH, Chandigarh) Pure cultures of *Staphylococcus aureus*, *Streptococcus mutans*, developed in Nutrient broth and *L. acidophilus* in MRS broth, respectively, at 37° C, 24 h, were taken as inoculum. The dentifrices Colgate Strong Teeth, Pepsodent Close up, Colgate gel, Cibaca, Anchor (chemical synthetic) Babool Dabur red Meswak,

(herbal synthetic) Hexidine, Listerine, Triguard, Senquel, Wokadine, Tantum Betadine Sflo (Mouth rinse) were obtained from local market.

Antimicrobial activity by disk diffusion method

All dentifrices (2.0 gm) were dissolved in 10 ml sterile distilled water to give 200 mg ml⁻¹ concentration of stock solution, respectively. Half a milliliter of this stock containing 100 mg dentifrice was used for the assay of antibacterial activity. The assay was performed by disk diffusion method^[18]. The antibacterial activity was measured as size of zone of inhibition (in millimeter). Three replicates were maintained for each dentifrice, and the experiment was repeated thrice.

RESULT AND DISCUSSION

The results obtained in this study suggest differences among the tested dentifrices regarding antimicrobial properties. Each test comparing zones of inhibition amongst the oral bacteria, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Staphylococcus aureus* and *Candida albicans* was accompanied by the dentifrices exhibiting a range of effectiveness

The antimicrobial activity shown on the agar plates varied among the 16 dentifrice product. The positive control produce significantly size inhibition zone for all four microorganisms. Among the 16 test dentifrices most of the antimicrobial activity at 24 hrs at full strength, in tooth paste **Colgate total** produce larger zone of inhibition against all four microorganism. Colgate total shows maximum zone of inhibition i.e. 65.0 mm after 24 hrs against *Staphylococcus aureus*. **Pepsodent** produces smaller zone than Colgate total, but it also gives a good result with larger zone i.e. 60 mm after 24 hrs. While in mouth washes **Hexidine** produces larger zone of inhibition against all the four microorganisms. Hexidine shows maximum zone of inhibition i.e. 21.2 mm against *Staphylococcus aureus* which is equal to the standard (Chlorhexidine gluconate). While in mouth washes **Triguard** produces second largest zone of inhibition after the Hexidine. In Mouth wash Hexidine shows larger zone of inhibition i.e. 15.5 mm. All the dentifrice products were very effectively against *Staphylococcus aureus*, and than against *Streptococcus mutans*.

Table 2 Antibacterial activity of tooth pastes against microorganisms (Zone of inhibition of growth in mm, average of 3 readings)

Dentifrices	Streptococcus mutans		Staphylococcus aureus		Candida albicans		Lactobacillus acidophilus		Direct mouth flora		
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	
Synthetic tooth pastes	Colgate Strong Teeth	29.5	30.5	65.0	70.0	27.7	30.7	29.0	31.2	21.5	23.7
	Pepsodent	28.2	29.2	60.0	68.0	30.0	30.0	26.0	27.7	23.2	24.7
	Close up	20.7	24.5	24.3	25.2	22.0	22.3	19.0	22.2	16.0	17.7
	Colgate gel	27.7	28.2	24.5	24.7	25.0	25.2	25.5	28.0	20.7	21.2
	Cibaca	21.5	23.2	21.0	21.5	22.0	23.1	24.2	24.7	19.5	20.0
	Anchor	21.7	22.3	58.0	64.5	31.2	32.5	27.0	28.5	22.5	23.2
Herbal tooth pastes	Babool	17.1	18.1	20.0	22.0	21.0	21.0	20.0	20.7	15.7	16.2
	Dabur red	20.5	22.2	19.2	19.6	21.7	22.7	21.7	23.7	18.5	18.7
	Meswak	21.5	23.2	21.0	22.3	20.7	19.1	24.7	25.0	17.7	18.2
	Hexidine	14.0	18.5	20.5	21.2	14.5	15.2	21.5	22.0	14.5	15.5
Mouth washes	Listerine	0.0	0.0	0.0	0.0	0.0	0.0	10.0	10.5	10.7	11.3
	Triguard	13.7	14.7	18.2	18.5	11.7	13.0	17.6	23.0	12.5	13.2
	Senquel	7.0	7.3	11.0	11.5	0.0	0.0	7.8	8.0	7.5	8.1
	Wokadine	0.0	0.0	9.5	9.7	8.0	8.5	10.0	10.7	7.7	8.5
	Tantum	6.2	6.2	8.2	8.5	8.2	8.7	12.2	11.7	13.2	13.5
	Betadine	6.7	6.7	9.3	9.7	9.0	9.0	11.5	12.0	11.5	11.7
	Sflo	0.0	0.0	0.0	0.0	0.0	0.0	9.5	10.0	14.0	14.5
	Control CHG	16.5	17.5	20.7	21.2	15.2	15.8	19.7	20.5	14.7	15.9

Table 3 Minimum Inhibitory Concentration (MIC) against *S. aureus* zone of inhibition in mm

		Concentration								
		70%	40%	30%	20%	10%	5%	2%	1%	0.5%
Synthetic tooth pastes	Colgate Strong Teeth	49.3	45.7	44.8	42.0	40.8	37.8	36.7	34.5	32.8
	Pepsodent	45.7	44.3	41.8	38.4	35.7	33.5	32.5	30.9	28.5
	Close up	36.5	31.2	25.7	18.5	13.4	9.7	0.0	0.0	0.0
	Colgate gel	38.5	33.4	28.4	22.7	17.6	12.4	0.0	0.0	0.0
	Cibaca	44.3	39.7	35.8	33.0	31.8	26.8	23.9	21.7	18.1
Herbal tooth pastes	Anchor	40.3	39.7	37.2	35.0	34.5	34.2	32.6	30.0	29.6
	Babool	35.7	31.4	23.3	17.7	11.2	0.0	0.0	0.0	0.0
	Dabur red	25.7	23.4	21.7	18.7	18.4	15.2	10.5	0.0	0.0
	Meswak	41.2	30.0	27.7	22.2	15.2	14.2	13.5	0.0	0.0

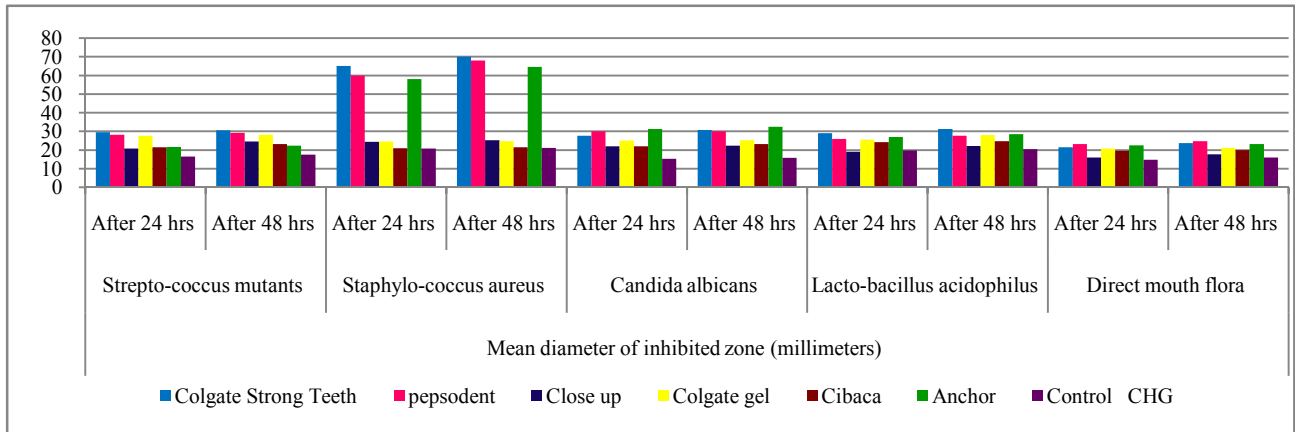


Fig 1 Synthetic tooth paste against all microorganism

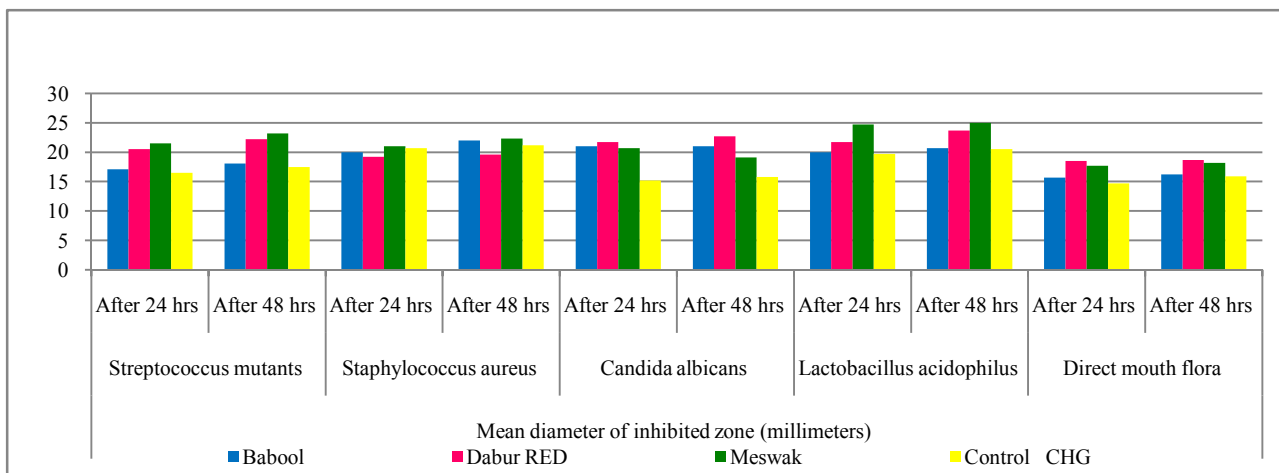


Fig 3 Herbal tooth paste against all microorganism:

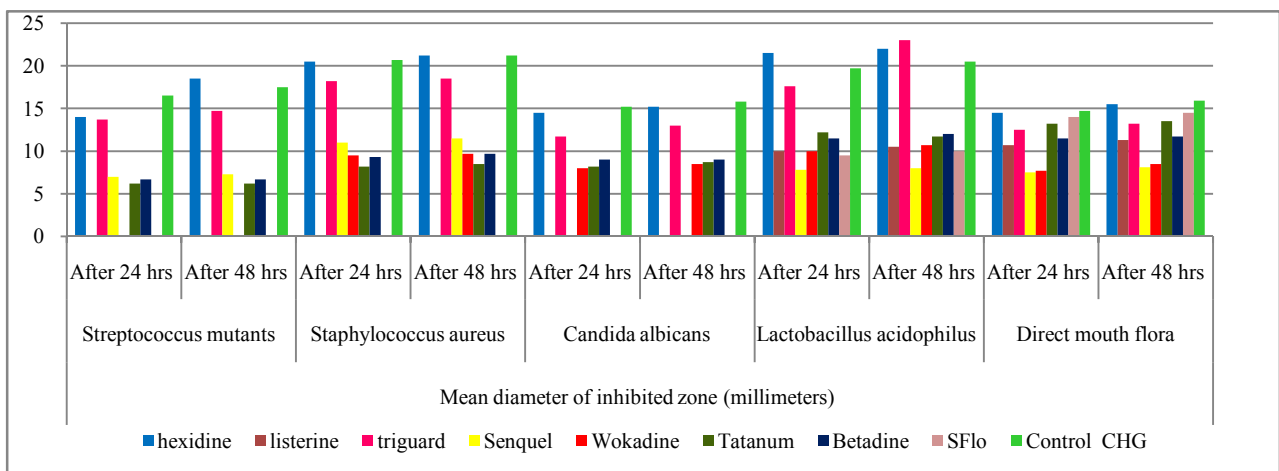
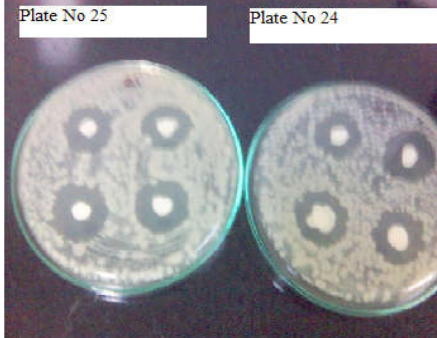



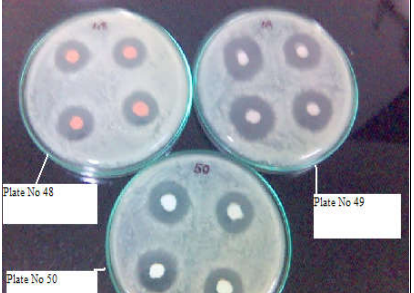
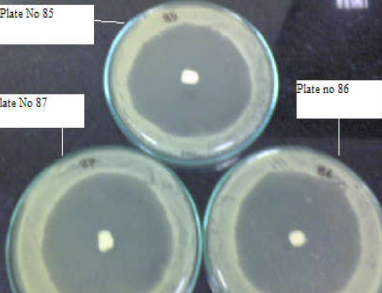

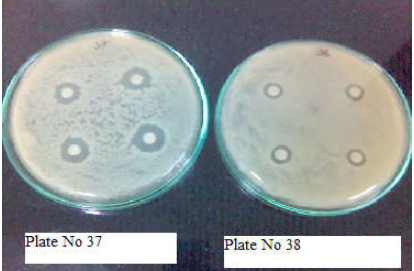
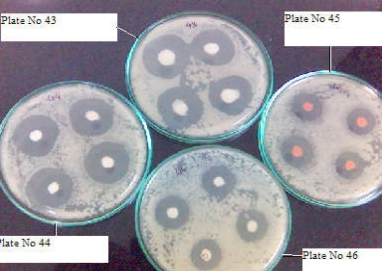


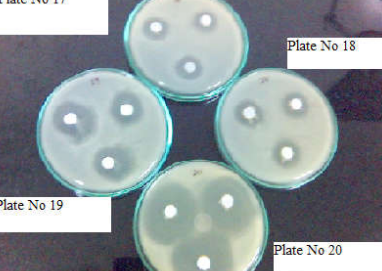


Fig 4 Mouth washes against all microorganisms

		
<p>Plate No 24, 25- Zone of inhibition respectively Meswak and Cibaca (tooth paste) against <i>S. mutans</i> on SCDA</p>	<p>Plate No 30- Zone of inhibition of Babool (tooth paste) against <i>S. mutans</i> on SCDA</p>	<p>Plate No 26, 27, 28, 29 Zone of inhibition respectively Anchor, Pepsodent, Dabur red, and Close up against <i>S. mutans</i> on SCDA</p>
		
<p>Plate No 62- Zone of inhibition of Betadine (mouth wash) against <i>S. aureus</i> on SCDA</p>	<p>Plate No 48, 49, 50- Zone of inhibition respectively Dabur red, Colgate gel, Cibaca (tooth paste) against <i>S. aureus</i> on SCDA</p>	<p>Plate No 85, 86, 87- Zone of inhibition respectively Colgate total, Pepsodent, Anchor (tooth paste) against <i>S. aureus</i> on SCDA</p>
		
<p>Plate No. 40,43,44 Zone of inhibition of Colgate Total, Anchor, Pepsodent against <i>C. albicans</i></p>	<p>Plate No. 37,38 Zone of inhibition of Hexidine and Triguard Pepsodent against <i>C. albicans</i></p>	<p>Plate No 45, 46- Zone of inhibition respectively Dabur red, Close up (tooth paste) against <i>C. albicans</i> on SCDA</p>
		
<p>Plate No 92- Zone of inhibition of Triguard (mouth wash) against <i>Lactobacillus acidophilus</i> on SCDA</p>	<p>Plate No 95, 96, 97- Zone of inhibition respectively Anchor, Colgate total, Pepsodent against <i>Lactobacillus acidophilus</i> on SCDA</p>	<p>Plate No 17, 18, 19, 20- Zone of inhibition respectively Meswak (tooth paste) 10%, 20%, 30%, 40% against <i>S. aureus</i> on SCDA</p>

But all the dentifrice products were not as effective against *Candida albicans* and *Lactobacillus acidophilus* as compare to other two organisms. **Anchor** shows good zone of inhibition against *Candida albicans*, but against other organisms (*Streptococcus mutans*, *Staphylococcus aureus*, *Lactobacillus acidophilus*) it shows smaller zone of inhibition.

The results obtained in this study suggest differences among the tested dentifrices regarding antimicrobial properties. Each test comparing zones of inhibition amongst the oral bacteria, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Staphylococcus aureus* and *Candida albicans* was accompanied by the dentifrices exhibiting a range of effectiveness. The antimicrobial activity shown on the agar

plates varied among the all dentifrice product. The positive control produce significantly size inhibition zone for all four microorganisms. Among the 16 test dentifrices it was observed that most of the antimicrobial activity at 24 hrs; detected little additional inhibition at 48 hrs. Colgate total produce larger zone of inhibition against all four microorganism. Colgate total shows maximum zone of inhibition i.e. 65.0 mm after 24 hrs against *Staphylococcus aureus*. While in mouth washes Hexidine produces larger zone of inhibition against all the four microorganisms. Hexidine shows maximum zone of inhibition i.e. 21.2 mm against *Staphylococcus aureus* which is equal to the standard (Chlorhexidine gluconate). Pepsodent produces smaller zone than Colgate total, but it also gives a good result with larger zone i.e. 60 mm after 24 hrs. While in mouth washes Triguard produces second largest zone of inhibition after the Hexidine. Anchor shows good zone of inhibition against *Candida albicans*, but against *Streptococcus mutans*, *Staphylococcus aureus*, *Lactobacillus acidophilus*. All the dentifrice products were very effectively against *Staphylococcus aureus*, and than against *Streptococcus mutans*. But all the dentifrice products were not as effective against *Candida albicans* and *Lactobacillus acidophilus* as compare to other two organisms. The effectiveness of all the dentifrice products against microorganisms is in this order; all the dentifrice products are very effective against *Staphylococcus aureus*, after that *Streptococcus mutans*, after that *Candida albicans* and in last against *Lactobacillus acidophilus*. It was found that, in tooth paste Pepsodent shows larger zone of inhibition i.e. 24.7 mm, while Colgate total also shows good result i.e. 23.7 mm. In Mouth wash Hexidine shows larger zone of inhibition i.e. 15.5 mm. The effectiveness of all dentifrice products is given below in descending order. In herbal products Meswak shows larger zone i.e. 42.2 mm at 70% concentration than other two herbal products i.e. Babool and Dabur red. The antimicrobial activity Commercial Dentifrices observed on the agar plates varied greatly among the all herbal dentifrices tested. The positive control produced significantly sized inhibition zones for all four microorganisms. The negative control produced no observable inhibitory effect. Among the chemical synthetic dentifrices, most of the dentifrices show significant the antimicrobial activity against *S. mutans* and *S. aureus*. Herbal dentifrices produced a significantly larger inhibition zone than the positive control. Therefore, the results for this dentifrice indicate chemical synthetic dentifrice found potent antibacterial agent help to reduce the oral infection cause by *Staphylococcus aureus*, *Streptococcus mutans*, *Lactobacillus acidophilus* and *Candida albicans*

CONCLUSION

The present study conclude that the commercial chemical synthetic dentifrices found excellent antibacterial activity, where as herbal dentifrices less antimicrobial potential against one or more of the four oral microorganisms. Further research efforts are needed to help establish guidelines to ensure the efficacy and safety of natural oral care products such as herbal dentifrices and mouth rinses

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