



***In vitro* Efficacy of Newly Formulated Oil Pulling against Oral Malodor Related Microbiota**

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PA and SC designed the study. Author SC managed the literature review, wrote the protocol, performed the statistical analysis and wrote the first draft of the manuscript. Authors SP and KP evaluated the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Oral malodor or bad breath is an unpleasant breath exhaled from the oral cavity and becomes physical, social and psychological impacts on affected individuals. Metabolic products, especially volatile sulfur compounds (VSCs) generated by oral microbiota are major causes of oral malodor. Swishing of oil pulling, an ancient protocol is used to keep oral health in good condition with fresh breath.

Aims: This study aims to examine an *in vitro* effectiveness against some oral microbes related to oral malodor of the oil pulling newly formulated by Dentiste.

Materials and Methods: The oil pulling Dentiste⁷M[®] by Dentiste was tested for the antimicrobial activity against *Streptococcus mutans* KPSK2, *Lactobacillus casei* ATCC393, *Porphyromonas gingivalis* ATCC33277, *P. gingivalis* W50 and *Candida albicans* ATCC10231 by agar disc diffusion and broth micro-dilution methods, respectively.

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Results: The growth of *P. gingivalis* W50 and ATCC33277 was strongly inhibited by this newly formulated oil pulling. Additionally, the diluted oil pulling (1:2) still expressed inhibitory effect against this bacterium predominantly related to oral malodor. Contrarily, the oil pulling showed no antimicrobial activity against other tested microorganisms.

Conclusion: This study has provided informative data to support the use of the oil pulling specially formulated by Dentiste as a primary prevention and management of oral malodor through the reduction of a VSC producing bacterium.

Keywords: Oral malodor; oil pulling; antimicrobial; oral health.

1. INTRODUCTION

Oil pulling or oil swishing is an ancient protocol that involves swishing of oil in the mouth to promote both oral and systemic health. Oil-based mouth rinses were widely used to prevent tooth decay, bleeding gum and oral malodor or bad breath as described in the traditional Indian folk remedies [1]. Globally, Oral Malodor (OM) affects most populations and causes social embarrassment, emotional and psychological distress and leads to the lack of self-esteem, self-image and self-confidence [2]. Types of OM are categorized into non-oral and oral causes according to site and etiology. Non-oral causes include physiological halitosis (caused by dietary foods and drinks); pathological halitosis (caused by some systemic diseases such as diabetes mellitus, kidney disease and gastritis with mucosal pathology) and psychological halitosis (delusional cacosomia). Other non-oral causes are ozostomia (causes from upper respiratory tract infections) and stomatodysodia (causes from lung infections). Interestingly, the commonest type of OM (over 90 percent) is oral causes so called fetor ex ore (FEO) [3].

Mature microbial biofilm with climax ecosystem, especially of odoriferous bacteria stagnated on teeth, gums and tongue is the origin of oral malodor. The commonest malodorous substances from oral biofilm metabolism are volatile sulfur compounds (VSCs), these emanating from climax ecosystems in periodontal pockets and pits on dorsal tongue surfaces. The main VSCs causing oral malodor include hydrogen sulfide (H_2S), methyl mercaptan (CH_3SH) and dimethyl sulfide ($(CH_3)_2S$), which are produced by catabolism of sulfur amino acids (cysteine and methionine) [4]. Many microorganisms produce VSC's, particularly gram-negatives, from mature stagnated oral biofilm generate oral malodor. These include *Porphyromonas gingivalis*, *Tannerella forsythia*, *Fusobacterium nucleatum*,

Klebsiella pneumonia, *Veillonella atypica*, *Prevotella intermedia* and *Treponema denticola*, species commonly isolated from OM patients [5].

There are reports relating a positive correlation between oral malodor and pathogenic oral states including poor oral hygiene, dental caries, gingivitis and periodontitis [2,4,6,7]. Increased oral hygiene procedures, which include brushing and flossing, tongue cleaning, mouth rinsing and oil swishing, are all recommended as effective therapy for oral malodor by disrupting biofilm stagnation to reduce microbial accumulation, especially H_2S -producing bacteria [8,9]. Dentiste'7M ® oil pulling is a newly formulated oil-based mouth rinse intended to moderate oral malodors. Accordingly this study analyzed *in vitro* the effects of Dentiste'7M ® (by Dentiste, Thailand), against OM-producing oral microorganisms.

2. MATERIALS AND METHODS

2.1 Research Study

Brain heart infusion (BHI) broth, BHI agar, and Sabouraud dextrose agar (SDA) were purchased from BD Difco™ (Becton, Dickison and Company, Sparks, MD, USA). Anaerobe basal broth and agar was bought from Oxoid (Oxoid LTD, Basingstoke, Hampshire, England). Dimethyl sulfoxide was purchased from Sigma-Aldrich® (S.M. Chemical Supplies Co. Ltd., Bangkok, Thailand). Chlorhexidine mouthwash (0.5% and 0.2%) was provided by the Faculty of Dentistry, Mahidol University. *Streptococcus mutans* KPSK2, *Porphyromonas gingivalis* W50 and *Candida albicans* ATCC10231 were kindly provided by the Department of Oral Microbiology, Faculty of Dentistry, Mahidol University. *Lactobacillus casei* ATCC393 and *P. gingivalis* ATCC33277 were purchased from Microbiologics® (Microbiologics, Inc., St. Cloud, MN USA). Flat-bottom 96 well plates were purchased from Nunc™ (Roskilde, Denmark).

Dentiste'7M ® oil pulling was provided by Dentiste, Thailand.

Each tested microorganism was freshly sub-cultured on appropriate agar plate and incubated in 37°C with 5% CO₂ incubator, except *P. gingivalis* ATCC33277 and W50 being incubated anaerobically at 37°C. Newly formulated oil pulling named Dentiste'7M was primarily examined for the antimicrobial activity by Kirby-Bauer antibiotic testing [10]. The oil was then determined for minimum inhibitory concentration (MIC) against the tested microorganisms using broth micro-dilution method with some modification [11]. Briefly, few colonies of fresh microbial subculture were suspended in BHI broth, except *P. gingivalis* being suspended in an anaerobe basal broth instead. All microbial suspensions were adjusted to yield 1x10⁷ organisms/ml approximately. Oil pulling was serially two-fold-diluted with 1% DMSO in culture medium (ranging from undiluted, diluted 1:2 to 1:512). Chlorhexidine mouthwash and 1% DMSO in culture medium were used as positive and negative controls, respectively. A 180 µl of oil pulling dilutions was plated individually into columns 1 to 10 while an identical amount of negative and positive controls was seeded in columns 11 and 12, respectively. Then a 20 µl of microbial suspension was added into each well. The plate was incubated aerobically with 5% CO₂, except *P. gingivalis* that was incubated anaerobically, for 48 h. MIC was evaluated from the lowest concentration of oil pulling capable of inhibiting the growth of certain tested microorganisms. Each test was performed in triplicate. All experiments were carried out for five different time periods.

2.2 Statistical Analysis

No statistical analysis was performed in this study. Antimicrobial activity of Dentiste'7M ® oil pulling against all tested microorganisms was described in terms of mean value of inhibition zone diameter (mm) with standard deviation.

3. RESULTS

The Dentiste'7M ® oil pulling only demonstrated an impressive antimicrobial activity against both reference strains of *P. gingivalis* (W50 and ATCC33277) by agar disc diffusion method as shown in (Fig. 1). In addition, the MIC of the oil pulling against this anaerobic bacterium was 1:2

determined by broth micro-dilution method. The inhibitory effect of this oil pulling against all tested microorganisms was summarized in (Table 1).



Fig. 1. Antimicrobial activity of Dentiste'7M ® oil pulling (D'7M), diluted Dentiste'7M ® [D'7M (1:2)], chlorhexidine mouthwash (CHX) (positive control), and dimethyl sulfoxide (DMSO) (negative control) against *P. gingivalis* ATCC33277 determined by agar disc diffusion method

4. DISCUSSION

Oral malodor is mainly originated from the accumulation of microbiota, especially the volatile sulfur compound (VSC) producers in saliva, dental plaque, and dorsal tongue surfaces. *P. gingivalis*, *Tannerella forsythia* and/or *Treponema denticola* are three bacterial species largely producing VSCs through the proteolytic activity [12]. Oil pulling has been used as an effective protocol to remove the bacteria, viruses and protozoa from the mouth for years. The oils have been recognized as antioxidants to damage microbial cell wall, particularly anaerobic bacteria. They then attract the lipid layer of bacterial cell membrane and pull to the oils through emulsification process [12]. Oil-coated teeth and gingiva inhibits bacterial co-aggregation and plaque formation *in vivo* [13-15]. Comparatively, there have been a few *in vitro* studies regarding to the antimicrobial activity of oil pulling against pathogenic oral microorganisms, especially dental caries causing bacteria [16,17]. The study here is probably one of few reports regarding *in vitro* inhibitory effect of oil pulling against OM-producing oral microbiota.

Table 1. Inhibitory effect of Dentiste'7M ® oil pulling newly formulated by Dentiste (Thailand) against oral malodor related microorganisms determined by agar disc diffusion method

Tested agents	Inhibition zone [mean of diameter (mm) ± standard deviation (SD)]				
	<i>S. mutans</i> KPSK2	<i>L. casei</i> ATCC393	<i>P. gingivalis</i> W50	<i>P. gingivalis</i> ATCC33277	<i>C. albicans</i> ATCC10231
Oil pulling	-	-	17±1.51	15.6±0.58	-
0.2% CHX	29.75±2.43	20.25±0.46	22.75±2.96	22.17±0.75	17.63±2.2
1.0% DMSO	-	-	-	-	-

(-): no inhibition zone appearance, CHX: chlorhexidine mouthwash, DMSO: dimethyl sulfoxide

P. gingivalis as mentioned above is a prominent bacterium producing VSCs and has a positive relationship with oral malodor and periodontitis [18]. Previously, effectiveness of oil pulling as a primary procedure to promote oral health and to moderate oral malodor has been analyzed through clinical indices (modified gingival index score, plaque index score, organoleptic breath assessment score, self-assessment of breath score); chemical index [BANA (enzymatic breakdown of N-benzoyl-dL-arginine-2-naphthylamide) test scores]; and microbiological index (total counts of aerobic and anaerobic bacteria) [13-15]. In current study, the Dentiste'7M ® oil pulling (by Dentiste, Thailand) illustrated potent inhibitory efficacy against both reference strains of *P. gingivalis* (W50 and ATCC32277) compared to chlorhexidine mouthwash (positive control). Additionally, the diluted oil pulling (1:2) still demonstrated the anti-*P. gingivalis* activity. It clearly showed that in addition to physicochemical properties of oil pulling to disturb bacterial aggregation and plaque formation, the Dentiste's7M ® oil pulling was fully capable of inhibiting or killing the planktonic form of this OM-related bacterium. With prolonged and forceful mechanical action (swishing), the oil may play a part in dislodging the sessile bacteria from the deep crevices within the mouth. Furthermore, *P. gingivalis* is a key producer of VSCs that are toxic to periodontal tissues even when present at extremely low concentration [19]. Long-term use of chlorhexidine mouthwash is not recommended due to some side-effects including brownish discoloration of teeth and dorsum of the tongue, taste perturbation, oral mucosal ulceration [20]. Subsequently, Dentiste'7M ® oil pulling (specially formulated by Dentiste, Thailand) may be proposed as a primary effective solution not only for oral malodor but also for periodontitis prevention and management when the inhibitory effect against the biofilm form is further analyzed.

However, the Dentiste'7M ® oil pulling was unable to inhibit the growth of dental caries causing bacteria (*S. mutans*) as well as oral

candidiasis causing fungus (*C. albicans*). The finding was not associated with previous reports demonstrated that other formulas of oil pulling inhibited the growth of these microorganisms [17,21,22]. None of oil pulling so far contained inhibitory effect on another cariogenic bacterium as *L. casei*. Based on current data, it means this newly formulated oil pulling, Dentiste'7M ® may be inappropriate to be used to battle against dental caries and oral candidiasis.

5. CONCLUSION

Dentiste'7M ® oil specially formulated by Dentiste, Thailand contains *in vitro* inhibitory effect against the prominent VSC producing bacterium, *P. gingivalis*. Consequently, swishing with this newly formulated oil may be benefit to assist in managing oral malodor.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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