ผลของน้ำมันมะพร้าวต่อปริมาณแบคทีเรียทั้งหมดและเชื้อสเตรปโตคอกคัสมิวแทนส์ในน้ำลาย

Effect of Coconut Oil on Salivary Total Bacterial and *Streptococcus Mutans* Counts

ดรุณี โอวิทยากุล[1], กาญจนา ปาลี[2], สาครรัตน์ คงขุนเทียน[3], เพ็ญพิชชา วนจันทรรักษ์[4]
[1]ภาควิชาทันตกรรมครอบครัวและชุมชน คณะทันตแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
[2]แผนกทันตกรรม โรงพยาบาลขุนตาล จังหวัดเชียงราย
[3]ภาควิชาทันตกรรมบูรณะและปริทันตวิทยา คณะทันตแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
[4]ปฏิบัติการวิจัยทางทันตแพทยศาสตร์ คณะทันตแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่
Darunee Owittayakul[1], Kanjana Palee[2], Sakornratana Khongkhunthian[3], Phenphichar Wanachantararak[4]
[1]Department of Family and Community Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai
[2]Dental Department, Khuntan hospital, Chiang Rai
[3]Department of Restorative Dentistry and Periodontology, Faculty of Dentistry, Chiang Mai University, Chiang Mai
[4]The Dental Research Center, Faculty of Dentistry, Chiang Mai University, Chiang Mai

ชม. ทันตสาร 2561; 39(1) : 75-83
CM Dent J 2018; 39(1) : 75-83

บทคัดย่อ

วัตถุประสงค์: เพื่อศึกษาผลของน้ำมันมะพร้าวบริสุทธิ์ต่อการลดปริมาณเชื้อแบคทีเรียทั้งหมด และสเตรปโตคอกคัส มิวแทนส์ในน้ำลาย โดยเปรียบเทียบกับน้ำยาบ้วนปากคลอเฮกซิดีนร้อยละ 0.12

วิธีการศึกษา: นักศึกษาระดับปริญญาตรี คณะทันตแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ จำนวน 40 คน แบ่งเป็น 2 กลุ่ม คือ กลุ่มควบคุม (คลอเฮกซิดีน กลูโคเนต ร้อยละ 0.12) และกลุ่มทดลอง (น้ำมันมะพร้าวบริสุทธิ์) ให้อาสาสมัครใช้น้ำยาบ้วนปาก 14 วัน และแปรงฟันตามถนัด ไม่มีการสอนวิธีการดูแลอนามัยช่องปากเพิ่มเติม ตรวจใน

Abstract

Objective: To investigate the effect of coconut oil in reducing the levels of total bacteria and *Streptococcus mutans* in saliva, and to compare its efficacy with that of 0.12% chlorhexidine mouthrinse.

Materials and Methods: Forty healthy undergraduate dental students at Chiang Mai University were randomly allocated into two groups, a control group (0.12% chlorhexidine gluconate) and an experimental group (coconut oil). All subjects were instructed to use the mouthrinses every day for

Corresponding Author:

ดรุณี โอวิทยากุล
ภาควิชาทันตกรรมครอบครัวและชุมชน
คณะทันตแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่

Darunee Owittayakul
Lecturer, Department of Family and Community Dentistry,
Faculty of Dentistry, Chiang Mai University,
Chiang Mai, 50200, Thailand
E-mail: ***jeeleeja@gmail.com***

ช่องปากและเก็บน้ำลาย ณ วันแรก และเมื่อใช้น้ำยาบ้วนปาก 14 วัน เพื่อตรวจหาจำนวนเชื้อแบคทีเรียทั้งหมดและสเตรปโตคอคคัส มิวแทนส์ ในน้ำลายในอาสาสมัครทั้ง 2 กลุ่ม

ผลการศึกษา: มีอาสาสมัคร 35 คน ใช้น้ำยาบ้วนปากครบตามระยะเวลา พบว่าคลอเฮกซิดีนลดปริมาณเชื้อแบคทีเรียทั้งหมดได้ร้อยละ 53 และ สเตรปโตคอคคัส มิวแทนส์ได้ร้อยละ 60 สูงกว่าน้ำมันมะพร้าว ซึ่งลดปริมาณเชื้อแบคทีเรียทั้งหมดได้ร้อยละ 45 และ สเตรปโตคอคคัส มิวแทนส์ได้ร้อยละ 39 แต่ไม่พบความแตกต่างอย่างมีนัยสำคัญระหว่างน้ำยาบ้วนปากสองชนิด

บทสรุป: การใช้น้ำมันมะพร้าวอมกลั้วปาก 2 สัปดาห์ สามารถลดปริมาณแบคทีเรียทั้งหมด และเชื้อสเตรปโตคอกคัส มิวแทนส์ในน้ำลาย ไม่แตกต่างจากน้ำยาบ้วนปากคลอเฮกซิดีนร้อยละ 0.12 ดังนั้นน้ำมันมะพร้าวบริสุทธิ์จึงเป็นทางเลือกหนึ่งในงานทันตกรรมป้องกันสำหรับใช้ดูแลอนามัยช่องปากได้

คำสำคัญ: คลอเฮกซิดีน น้ำมันมะพร้าว น้ำยาบ้วนปากออยล์พูลลิ่ง เชื้อสเตรปโตคอคคัส มิวแทนส์

two weeks. They were advised to perform their routine oral hygiene practice without any additional education. Oral examination and saliva collection were performed at baseline and day 14. Total bacterial and *S. mutans* counts in saliva were measured and compared between two groups.

Results: Thirty-five subjects in the coconut oil group completed the study. The percentage reduction in total bacterial count was 53% for chlorhexidine and 45% for coconut oil. The percentage reduction in *S. mutans* count was 60% for chlorhexidine and 39% for coconut oil. However, there was no significant difference between the two mouthrinses.

Conclusions: Two weeks of coconut-oil pulling showed a similar percentage reduction in total bacterial and *S. mutans* count to that produced by 0.12% CHX mouthrinse. Thus, coconut oil can also be an alternative mouthrinse in preventive therapy to maintain oral hygiene.

Keywords: chlorhexidine, coconut oil, mouthrinse, oil-pulling, streptococcus mutans

Introduction

Periodontal diseases and dental caries are major oral health problems and are related to dental plaque biofilm.[1] "*Streptococcus mutans*" is the pathogen (most strongly) associated with dental caries.[1] Mechanical plaque control is effective in removing supragingival plaque in areas where access to plaque deposits is possible.[2] Antimicrobial mouthrinses are used as an adjunct method to reduce biofilm build up on soft tissue surfaces in the oral cavity, and potentially delaying plaque accumulation over teeth.[3]

Chlorhexidine gluconate (CHX) is considered as the "gold standard" mouthrinse. It is a broad-spectrum antimicrobial agent and is widely used to prevent periodontal diseases because it is the most effective antimicrobial, antiplaque and antigingivitis agent currently approved.[4] It is also a potent substance for dental caries prevention.[5] The disadvantages of CHX are unpleasant taste and mucosal irritation.[6] Prolonged use of CHX mouthrinse can cause tooth staining, increase calculus formation and alteration of taste perception.[6] Therefore, a new or alternative mouthrinse with antibacterial activity and fewer side effects from natural products has been studied.

Oil-pulling or oil-swishing or therapy with edible oils is used primarily in Ayuravedic medicine. This

procedure is simply done by swishing a tablespoon of oil in the mouth for 10 - 20 minutes.[7] It is claimed that the procedure has benefits for the prevention of oral diseases, including dental caries, oral malodor, bleeding gums and cracked lips.[8] Vegetable oils, such as sunflower oil, sesame oil or coconut oil are becoming increasingly of interest for use as an alternative antimicrobial mouthrinse. An *in-vitro* study showed that sesame oil has antibacterial activity against *S. mutans*, sunflower oil has antifungal activity against *C.albicans*, whereas coconut oil exhibits antimicrobial activity against both *S.mutans* and *C.albican*s.[9]

Coconut oil in Thailand is commercially available in local markets. It is a plentiful source of useful medium chain fatty acids (MCFAs), particularly, lauric acid (nearly 50% of coconut's fat content), capric acid, caprylic acid and caprioic acid[10] which exhibit bacterial inhibition.[11] The bactericidal activity of monocaprin, capric acid, and lauric acid on streptococci and staphylococci is effective 10 minutes after treatment.[12] However, there have been few clinical trials showing the effect of coconut oil on oral bacteria, especially *S. mutans*. Moreover, no study has reported the effect of coconut oil on the total colony count of oral microorganisms.

The purposes of this study were to evaluate the efficacy of coconut oil-pulling in reducing the levels of total bacteria and *S. mutans* in saliva compared to 0.12% CHX mouthrinse.

Materials and methods

Ethics

The study was reviewed and approved the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University (Document No.26/2558) and informed consent was obtained after the potential risks and benefits of participation were explained to the participants.

Study sample

Forty participants were recruited from 2nd to 6th year undergraduate dental students of Chiang Mai University, Thailand. The subjects were selected based on the following selection criteria:

Inclusion criteria

Healthy dental students aged 18-25 years, presenting with mild to moderate gingivitis according to gingival index (GI) of Löe and Silness[13] and had having at least 20 natural permanent teeth, excluding third molars.

Exclusion criteria

1. Subjects with any systemic diseases or salivary gland disorders
2. Pregnant or breast-feeding women
3. Smokers
4. Subjects having orthodontic or prosthodontic appliances.
5. Subjects having taken antibiotics or any other drugs within three months prior to enrollment in the study or during the study period.
6. Subjects having used any mouthrinse within a month prior to enrollment.
7. Subjects with previous use of lozenges that have anti-microbial effects at least one month prior to enrollment or during the study period.
8. Subjects having a history of allergy to coconut oil, CHX or any of the ingredients used in the study treatment.

Procedure for gathering data

This *in vitro* study was conducted between November, 2015 and October, 2016. Prior to the study, all subjects were interviewed about their demographic characteristics, medical history, dental history and oral hygiene practices. Oral examination was performed and saliva samples were collected at baseline and on

day14. Forty participants were randomly allocated to one of the two groups according to mouthrinses:

1. Experimental group (n=20): Virgin coconut oil (Coco Delight®, GPO, Bangkok, Thailand) (lot: NP580021) was used. Fifteen milliliters of coconut oil was kept in the mouth and swished between the teeth for 10 minutes at night after tooth brushing.

2. Control group (n=20): 0.12% CHX mouthrinse (Manufactured by Faculty of Dentistry, Chiang Mai University, Chiang Mai, Thailand) was used. Fifteen milliliters of CHX was rinsed for one minute twice a day after meals.

All subjects were instructed to use the mouthrinses every day for two weeks and to refrain from drinking or eating for 30 minutes after using the oral rinses. They were advised to perform their routine oral hygiene practice without any additional education but with emphasis on following the mouthrinsing instructions strictly. The same brands of toothbrushes (Colgate slim soft, Colgate-Palmolive, Bangkok, Thailand) and toothpastes (Colgate total pro gum health, Colgate-Palmolive, Bangkok, Thailand) were given to all subjects to eliminate the effects of the different toothbrushes and the compositions of different toothpastes. After one week, the subjects were recalled to evaluate any adverse effects and to ensure that they continued using the mouthrinses. They were also instructed to report back with the empty bottles to check their compliance. On the 14th day of the study, oral examination was performed by one researcher and saliva samples were collected by a different researcher. Both researchers were blind to the type of mouthrinse being used.

Saliva collection

Unstimulated whole saliva samples were collected in the morning between 10.00 am and 12.00 noon.[14] The subjects were instructed to avoid food and drink for at least two hours before saliva collection. Five milliliters of whole saliva were collected in a sterile tube for up to 10 minutes. The saliva tubes were immediately placed on ice and transferred to the microbiological laboratory for processing within two hours after saliva collection.

Determination of microorganisms

Saliva samples were vortexed and 10-fold serially diluted with sterile phosphate buffer solution (PBS). The diluted saliva samples were spread on brain heart infusion (BHI) agar (Merck, Darmstadt, Germany) and modified-sucrose bacitracin culture medium (SB-20M agar) to determine total bacterial and *S. mutans* counts, respectively. The compositions of agar culture media used in this study are shown in Table 1. Each diluted suspension was cultured in triplicate. The BHI agar plates were incubated in an aerobic incubator chamber (Memmert®, Memmert GmbH + Co. KG, Schwabch, Germany) at 37°C for 48 hours. The SB-20M agar plates were placed in an anaerobic incubator (Bactron®, Sheldon, Oregon, USA) with 5% CO_2 at 37°C for 72 hours. After incubation, the number of colonies was counted, using ImageJ software (U.S. National Institutes of Health, Bethesda, Maryland, USA), only on the most suitable plates (those with 30–300 colonies). The colonies were expressed as colony-forming units per ml of saliva (CFU/ml) and determined at baseline and on day14.

Statistical analysis

Total bacteria and *S. mutans* counts were performed statistically using SPSS 17.0 software (SPSS, Inc., Chicago, Illinois, USA). Standard error was analyzed from triplicate data. The percentage reduction in total bacterial and *S. mutans* counts between baseline and day 14 were calculated for the two mouthrinses and compared between the two groups

ตารางที่ 1 *ส่วนประกอบของอาหารเลี้ยงเชื้อที่ใช้ในการศึกษานี้*

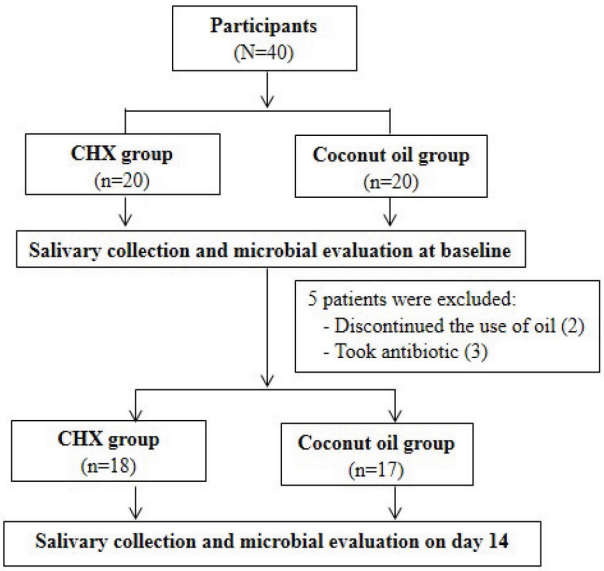
Table 1 *Compositions of agar culture media used in this study.*

Media	Constituents per liter of culture medium agar
BHI	Brain Heart Infusion agar; BHI (agar base; Merck, Darmstadt, Germany)
SB-20M	Bacto-casitone (15.0 g); yeast extract (5.0 g); cysteine (0.2 g); sodium sulphite (0.1 g); Sodium acetate (20.0 g); coarse granular cane sugar (200.0 g); agar (15.0 g); distilled water (qsp); and bacitracin added to a final concentration of 0.2 U/mL agar.

using the independent samples t-test. A significance level of $p < 0.05$ was set.

Results

Thirty-five (14 males and 21 females) of 40 subjects in the age range 20 to 25 years completed the study (Figure 1). There were 17 pre-clinical students (48.6%) and 18 clinical students (51.4%). Five subjects were excluded from the study, as two subjects discontinued the use of the oil and three subjects took antibiotics during the study period. The average age of the all participants was 22.31±1.81 years. The distribution of age, gender and educational level as well as average bacterial counts at baseline (BL) between the two groups were not statistically significantly different (Tables 2 and 3).



รูปที่ 1 *ขอบเขตของงานวิจัย*

Figure 1 *Scope of the study*

ตารางที่ 2 *ข้อมูลทั่วไปของอาสาสมัคร*

Table 2 *Demographic characteristics of subjects.*

Data		Group				p-value
		012% CHX (N = 18)		Coconut oil (N = 17)		
Mean age ±SD (years)		22.3±1.90		22.1±1.80		0.627[a]
Gender N (%)	Male	8	44.40	6	35.30	0.862[b]
	Female	10	55.60	11	64.70	
Educational level N (%)	Pre-clinic	8	44.40	9	52.90	0.740[b]
	Clinic	10	55.60	8	44.40	

a=independent samples t- test; b=chi-square test

ตารางที่ 3 *ค่าเริ่มต้นก่อนการศึกษาและค่าเฉลี่ยร้อยละการลดลงของปริมาณแบคทีเรียทั้งหมด และเชื้อสเตรปโตคอคคัสมิวแทนส์*

Table 3 *Baseline data and average percentage reduction in total bacterial and S. mutans counts.*

Strains	Group		
	0.12%CHX (N=18)	Coconut oil (N=17)	*p*-value[a]
Total bacteria			
Mean BL ±SE (×10^2 CFU/mL)	89.1±26.81	35.6±9.63	0.074
% Reduction (mean±SE)	53.2±10.1	44.9±9.2	0.548
S. mutans			
Mean BL ±SE (×10^2 CFU/mL)	26.1±11.63	12.8±4.19	0.294
% Reduction (mean±SE)	60.6±9.9	39.2±10.4	0.145

a=independent samples t-test

After a two-week regimen of rinsing with both mouthrinses, the total bacterial and *S. mutans* counts declined. Average percentage reduction in total bacterial and *S. mutans* counts in the CHX group was greater than that in the coconut oil group as presented in Table 3.

The results revealed that 0.12% CHX showed higher efficiency in bacterial reduction than did coconut oil. However, no statistically significant differences were noted in the percentage reduction in total bacterial and *S. mutans* counts between the two mouthrinses (p=0.548 and 0.145, respectively), as shown in Table 3.

Discussion

Coconut oil had efficiency in reducing *S. mutans* levels is supported by *in vivo* studies.[14,17,18] The results of this study showed that the average percentage reduction in total bacterial and *S. mutans* levels were not statistically different between coconut oil and 0.12% CHX mouthrinse. A recent study by Singla *et al*.[17] found that the percentage reduction of *S. mutans* after 10 minutes of gum massage for three weeks with either coconut oil or 1% CHX gel (Hexigel) was not significantly different. The median percentage reduction in *S. mutans* counts were 33.8% for coconut oil and 45.4% for CHX, values which were less than those in this study. However, sample size of each group evaluated in their study was small (n=8), and the population and methods were different from those in our study. Singla *et al*.[17] conducted their study on hospital housekeepers in rural regions in India, aged 18-55 years who had low education, low incomes and were unable to access dental care. They indicated that gum massage with oil is easier to perform and can have better patient compliance than swishing oil in the mouth for 10-15 minutes. That technique can be used in patients with special needs who are unable to control swallowing. Moreover, gingival massage has a beneficial role in mechanical disruption of the biofilm on the teeth and stimulation of gingival blood circulation.[17] Peedikayil *et al*.[18] also showed that the *S. mutans* levels in salivary and plaque samples from patients who swished with coconut oil were significantly decrease on day 15 and day 30 compared to the baseline. Furthermore, the *S. mutans* levels were not significantly different between coconut oil and 2% CHX mouthrinses at day 30. That study was conducted in 50 girls aged 8-12 years. They performed oil-pulling every day in the morning for two to three minutes under the observation of a caregiver. It was noteworthy that three

minutes of coconut-oil pulling was effective in reducing *S. mutans* levels in 15 and 30 days. Kaushik *et al*.[14] studied 60 healthy dental students in India aged 18-22 years and found that there was a significant reduction in *S. mutans* counts in saliva after 10 minutes of coconut-oil pulling and one minute of 0.2% CHX rinsing per day for two weeks. The reduction in the mean *S. mutans* counts was greater with CHX than with coconut oil. The percentage change of *S. mutans* counts were 22.79% for coconut oil and 25.72% for CHX. However, there was no statistically significant difference between coconut-oil pulling and 0.2% CHX mouthrinse. This lack of difference might result from the small sample size in each group (n=20).

CHX mouthrinses are available in concentrations of 0.2% and 0.12%. Its mechanism of action is that the cationic molecule binds to the negatively-charged cell walls of the microbes, causing membrane disruption and cell death.[19] A previous study encouraged prescription of 0.12% CHX because the lower concentration of CHX has less side effects and 0.2% CHX do not seem to be more effective against plaque and gingivitis.[20] Furthermore, two weeks of rinsing with 0.12% CHX mouthrinse produces a significant reduction in *S. mutans* counts.[21] Therefore, our study used 0.12% CHX mouthrinse and restricted the study period to 14 days to prevent tooth staining associated with prolonged usage of CHX mouthrinse.

The mechanism of action of oil-pulling is still not clear.[7] The mechanism may be that viscosity of the oil possibly inhibits bacterial adhesion and plaque coaggregation.[22] It has been hypothesized that monolaurin, the monoglycerides of lauric acid, and other medium chain monoglycerides contained in coconut oil have the capacity to alter bacterial walls, penetrate and disrupt cell membranes, inhibit enzymes involved in energy production and nutrient transfer, resulting in bacterial death.[23] Furthermore, MCFAs, such as capric acid, and lauric acid, are broadly inhibitory of bacteria.[11] Many studies have demonstrated that monolaurin from coconut oil has antimicrobial activity against various Gram-positive and Gram-negative organisms.[23-25] Another possible mechanism may be saponification, or the 'soap-making' process. This process is the result of alkaline hydrolysis of fat when the alkali in the saliva reacts to the oil.[14,18] Coconut oil has a high saponification value and is a commonly-used oil in making soaps.[18] Soap is considered as a good cleaning agent because it is an effective emulsifier.[14] Moreover, the oil swishing exerts mechanical shear forces, causing emulsification, by which insoluble fats in the oil are broken down into droplets, which disperse in water, leading to increased surface area of the oil and increased cleansing action.[14,18]

The adverse effects of coconut-oil pulling have not been reported. The subjects in a previous study did not complain of any kind of discomfort, taste alteration, burning sensation, or any other adverse effects after using coconut oil for two weeks, and most of them wished to continue using it.[17] Moreover, another study demonstrated that no staining was observed in the subjects using coconut oil, whereas a few subjects using 0.2% CHX mouthrinse revealed mild tooth staining.[18] Nevertheless, a few case reports have shown that the aspiration of oil during oil-pulling can cause lipoid pneumonia.[26,27]

In this study, two subjects in the coconut oil group were excluded because they discontinued using the oil. A main drawback of coconut oil-pulling was the required long rinsing duration, causing termination of mouth rinsing before the required time of 10 minutes had elapsed. However, Peedikayil *et al*.[18] demonstrated that daily coconut oil-pulling for two to three minutes after tooth brushing results in a statistically significant reduction in *S. mutans* counts. Therefore, coconut oil

could be used as a mouthrinse for a shorter rinsing duration. Further studies regarding the effect of shorter coconut oil-pulling duration on reducing the levels of oral microorganisms should be conducted. Traditionally, oil-pulling is performed in the morning time daily. This might be a problem for people who live busy lives. Therefore, this study prescribed oil-pulling at night time after tooth brushing to encourage the participants to follow the instructions strictly and consistently.

A limitation of this study was the small sample size and the differences in baseline data between the two groups. Future studies with large sample sizes and equal baseline data are needed.

Coconut oil is a natural extract and it also has a pleasing scent, good taste and fewer side effects. The results of this clinical study could be useful as scientific evidence to support swishing with coconut oil as an alternative mouthrinse in self-care and preventive therapy to maintain oral hygiene, especially in patients who have a history of allergy to CHX or need or wish to avoid the side effects of CHX mouthrinse. However, further studies are also needed to investigate the long-term effects of coconut-oil pulling on gingivitis, oral health and the patient's satisfaction.

Conclusions

Under the conditions of this *in vivo* study, it was revealed that two weeks of coconut oil pulling had similar a percentage reduction in total bacterial and *S. mutans* counts to 0.12% CHX mouthrinse.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

The authors would like to thank the Faculty of Dentistry, Chiang Mai University for financially supporting this study. We also thank Dr. Thanapat Sastraruji for his statistical advice and Dr. M. Kevin O Carroll, Professor Emeritus, University of Mississippi School of Dentistry, USA and Faculty Consultant, Faculty of Dentistry, Chiang Mai University, Thailand, for his assistance in the preparation of the manuscript.

References

1. Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. *Periodontol 2000* 2002; 28:12-55.
2. Gusberti FA, Sampathkumar P, Siegrist BE, Lang NP. Microbiological and clinical effects of chlorhexidine digluconate and hydrogen peroxide mouthrinses on developing plaque and gingivitis. *J Clin Periodontol* 1988; 15:60-67.
3. Teles RP, Teles FRF. Antimicrobial agents used in the control of periodontal biofilms: effective adjuncts to mechanical plaque control? *Braz Oral Res* 2009; 23:39-48.
4. Balagopal S, Arjunkumar R. Chlorhexidine: the gold standard antiplaque agent. *J Pharm Sci Res* 2013; 51:270-274.
5. Anderson MH. A review of the efficacy of chlorhexidine on dental caries and the caries infection. *J Calif Dent Assoc* 2003; 31:211-214.
6. Flötra L, Gjermo P, Rolla G, Waerhaug J. Side effects of chlorhexidine mouth washes. *Scand J Dent Res* 1971; 79:119-125.
7. Hebbar A, Keluskar V, Shetti A. Oil pulling–Unraveling the path to mystic cure. *J Int Oral Health* 2010; 2:11-15.
8. Asokan S. Oil pulling therapy. *Indian J Dent Res* 2008; 19:169.
9. Thaweboon S, Nakaparksin J, Thaweboon B. Effect of oil-pulling on oral microorganisms in biofilm models. *Asia J Public Health* 2011; 2:62-66.
10. DebMandal M, Mandal S. Coconut (Cocos nucifera L.: Arecaceae): in health promotion and disease

prevention. *Asian Pac J Trop Med* 2011; 4:241-247.

11. Huang CB, Alimova Y, Myers TM, Ebersole JL. Short- and medium-chain fatty acids exhibit antimicrobial activity for oral microorganisms. *Arch Oral Biol* 2011; 56:650-654.
12. Bergsson G, Arnfinnsson J, Steingrímsson O, Thormar H. Killing of Gram-positive cocci by fatty acids and monoglycerides. *Apmis* 2001; 109:670-678.
13. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand* 1963; 21:533-551.
14. Kaushik M, Reddy P, Sharma R, Udameshi P, Mehra N, Marwaha A. The effect of coconut oil pulling on *Streptococcus mutans* count in saliva in comparison with chlorhexidine mouthwash. *J Contemp Dent Pract* 2016; 17:38-41.
15. Wan AK, Seow WK, Walsh LJ, Bird PS. Comparison of five selective media for the growth and enumeration of *Streptococcus mutans*. *Aust Dent J* 2002; 47:21-26.
16. Saravia ME, Nelson-Filho P, Ito IY, da Silva LAB, da Silva RAB, Emilson C-G. Morphological differentiation between *S. mutans* and *S. sobrinus* on modified SB-20 culture medium. *Microbiol Res* 2011; 166:63-67.
17. Singla N, Acharya S, Martena S, Singla R. Effect of oil gum massage therapy on common pathogenic oral microorganisms-A randomized controlled trial. *J Indian Soc Periodontol* 2014; 18:441.
18. Peedikayil FC, Remy V, John S, Chandru TP, Sreenivasan P, Bijapur GA. Comparison of antibacterial efficacy of coconut oil and chlorhexidine on *Streptococcus mutans*: An *in vivo* study. *J Int Soc Prev Community Dent* 2016; 6:447-452.
19. Hugo WB, Longworth AR. The effect of chlorhexidine on the electrophoretic mobility, cytoplasmic constituents, dehydrogenase activity and cell walls of *Escherichia coli* and *Staphylococcus aureus*. *J Pharm Pharmacol* 1966; 18:569-578.
20. Najafi MH, Taheri M, Mokhtari MR, Forouzanfar A, Farazi F, Mirzaee M, *et al*. Comparative study of 0.2% and 0.12% digluconate chlorhexidine mouth rinses on the level of dental staining and gingival indices. *Dent Res J (Isfahan)* 2012; 9:305-308.
21. Kulkarni V, Damle S. Comparative evaluation of efficacy of sodium fluoride, chlorhexidine and triclosan mouth rinses in reducing the mutans streptococci count in saliva : an *in vivo* study. *J Indian Soc Pedod Prev Dent* 2003; 21:98-104.
22. Asokan S, Rathan J, Muthu MS, Rathna PV, Emmadi P. Effect of oil pulling on *Streptococcus mutans* count in plaque and saliva using Dentocult SM Strip mutans test: A randomized, controlled, triple-blind study. *J Indian Soc Pedod Prev Dent* 2008; 26:12-17.
23. Verallo-Rowell VM, Dillague KM, Syah-Tjundawan BS. Novel antibacterial and emollient effects of coconut and virgin olive oils in adult atopic dermatitis. *Dermatitis*. 2008;19(6):308-315.
24. Carpo BG, Verallo-Rowell VM, Kabara J. Novel antibacterial activity of monolaurin compared with conventional antibiotics against organisms from skin infections: an *in vitro* study. *J Drugs Dermatol* 2007; 6:991-998.
25. Ogbolu DO, Oni AA, Daini OA, Oloko AP. *In vitro* antimicrobial properties of coconut oil on *Candida* species in Ibadan, Nigeria. *J Med Food* 2007; 10:384-387.
26. Kim JY, Jung JW, Choi JC, Shin JW, Park IW, Choi BW. Recurrent lipoid pneumonia associated with oil pulling. *Int J Tuberc Lung Dis* 2014;18:251-252.
27. Kuroyama M, Kagawa H, Kitada S, Maekura R, Mori M, Hirano H. Exogenous lipoid pneumonia caused by repeated sesame oil pulling: a report of two cases. *BMC Pulm Med* 2015; 15:135.