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In-vitro antimicrobial effectiveness of herbal-based mouthrinses against oral microorganisms

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PEER REVIEW

Peer reviewer

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Comments

This is a valuable research work in which authors have demonstrated the antimicrobial activity of novel mouthwashes. The activity was assessed by the disk diffusion method.
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ABSTRACT

Objective: To evaluate the *in vitro* antimicrobial effectiveness of commercial herbal-based mouthrinses against oral microorganisms.

Methods: A total of three mouthrinses (OX, Pesona and Watsons) were tested for their antimicrobial activity against six oral organisms, *Streptococcus mutans* (*S. mutans*), *Streptococcus sobrinus* (*S. sobrinus*), *Lactobacillus salivarius* (*L. salivarius*), *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Candida albicans* (*C. albicans*) by standard agar-disk diffusion assay. Oradex mouthrinse containing 0.12% chlorhexidine gluconate and sterile distilled water was served as positive and negative controls, respectively.

Results: All mouthrinse formulations were effective in inhibiting the growth of *S. mutans*, *S. sobrinus*, *L. salivarius* and *C. albicans*. Among the tested mouthrinses, Pesona was the only effective mouthrinse against *S. aureus* and *P. aeruginosa*, similar to Oradex mouthrinse. Pesona mouthrinse formulation appears to be as effective as Oradex mouthrinse formulation to kill *S. aureus* and *P. aeruginosa*. Statistical analysis showed no significant difference among the tested formulations regarding their antimicrobial activities ($P > 0.05$).

Conclusions: Pesona was not the only herbal mouthrinse effective in inhibiting the growth of *S. mutans*, *S. sobrinus*, *L. salivarius* and *C. albicans in vitro*. All tested formulations were effective against those strains. Our findings may serve as a guide for selecting a kind of herbal mouthrinses as well as providing information to the dental professionals about the efficacy of these products.

KEYWORDS

Mouthrinses, Chlorhexidine gluconate, Antimicrobial properties, Zones of inhibitions

1. Introduction

Dental caries affecting preschool children have become a major health care problem in many developing countries including Malaysia. Dental caries are a pathological infectious disease that ends up in the destruction of hard dental tissue. Two bacteria species have been implicated in caries formation, namely, *Streptococcus mutans* (*S. mutans*) and lactobacilli. It has been shown that a process starting with the colonization of *S. mutans* and continuing with lactobacilli in susceptible individuals, leads to caries initiation and progression[1-3]. In addition, other microflora like *Streptococcus sobrinus* (*S. sobrinus*), *Staphylococcus aureus* (*S. aureus*),

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Pseudomonas aeruginosa (*P. aeruginosa*) and *Candida* spp are also associated with dental caries[2-5].

Mouthrinses are used in conjunction with mechanical oral hygiene such as brushing and flossing in order to prevent different kinds of oral diseases such as to treat high caries risk and infection, reduce inflammation, relieve pain and reduce halitosis. However, long-term use of ethanol-containing mouthrinses should be discouraged to patients with a high risk of caries. The widespread use of mouthrinses as an aid to oral hygiene has resulted in an increasing numbers of these products in the market. Development and the antimicrobial property assessment of the mouthrinses (apart from chlorhexidine) have been done mostly by the manufacturers, and little has been reported in the scientific literature[6-10]. Most of the work that has been published are related to the individual antiseptic ingredients rather than the complete formulation of mouthrinses.

Mouthrinses are available in a variety of compositions; many claims asserted to have antimicrobial properties, but very little research has been conducted to confirm these claims. At present, there is little information available on the antimicrobial potential of herbal-based mouthrinses made in Malaysia. Based on these considerations, we conducted an *in vitro* study to assess the efficacy of antimicrobial properties of three herbal-based mouthrinses formulation available on the market against bacteria and fungi which were commonly found in the oral cavity using agar-disk diffusion method.

2. Materials and methods

2.1. Test organisms

In the current study, four Gram-positive, one Gram-negative and one fungus were used to assess the antimicrobial activity of *S. mutans* (ATCC 35668), *Lactobacillus salivarius* (*L. salivarius*) (clinical isolate) and *S. sobrinus* (clinical isolate), which were obtained from the Craniofacial Laboratory, School of Dental Sciences, Universiti Sains Malaysia, Kelantan, Malaysia. The clinical isolates of *S. aureus*, *P. aeruginosa* and *Candida albicans* (*C. albicans*) were obtained from Medical Microbiology and Parasitology Laboratory, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia.

2.2. Collection of mouthwashes

Three different brands of herbal-based mouthrinses (OX, Pesona and Watsons) and Oradex antibacterial mouthrinse containing 0.12% chlorhexidine were purchased from local market. The particulars of ingredients including each mouthwash formulation by the manufacturers are listed in Table 1.

2.3. Determination of antimicrobial activity

Different formulations of herbal-based mouthrinses were tested for antimicrobial activity at full strength using agar-disk diffusion method. This method will determine the ability of antimicrobial agent of different herbal-based mouthwashes to inhibit formation of new bacterial and fungal colonies through the formation of inhibition zone. Each bacterium was suspended in 2 mL of peptone water whereas *C. albicans* was suspended in 2 mL of sterile saline. The turbidity of this suspension was adjusted to a 0.5 McFarland standard by using turbidimeter. A sterile cotton swab was dipped into the inoculum suspension and a lawn was made on Mueller-Hinton agar (*S. aureus*, *L. salivarius*, *P. aeruginosa* and *S. sobrinus*), Mueller-Hinton blood agar (*S. mutans*) and Sabouraud dextrose agar (*C. albicans*) plates. Sterile discs were impregnated with 80 μL of respective mouthwashes and applied on the surface of the agar using sterile forceps. Disc impregnated with 80 μL Oradex mouthwash and water was used as positive and negative controls, respectively. These plates were allowed to stand for 10 min for diffusion of the mouthwash to take place. These plates were then incubated at 37 ˚C for 24 to 48 h and the zone of inhibition was measured. This experiment was done in triplicates for each mouthwash. The diameter of the zone of inhibition (clear zone around each well) was measured with a digital caliper in millimeters. Data collection was performed on each plate after 24 to 48 h. The experiment was conducted in triplicates for each mouth rinse.

Table1
Ingredients of various herbal-based mouthrinses for antimicrobial activity.

Mouthwashes	Ingredients	Manufacturer
Pesona (fresh mint with tea tree oil)	Purified water, sorbitol, polyethylene glycol-40, sodium benzoate, sodium saccharin, methylparaben, cetylpyridinium chloride (CPC), eucalyptus oil, peppermint oil, mint, tea tree oil, citric acid, CI 42090, CI 19140	HCLC Sdn Bhd., Malaysia
OX herbal mouthwash	Aqua, glycerin, polyethylene glycol-40, hydrogenated castor oil, poloxamer 407, flavor, vera, peppermint oil, xylitol and green tea, seaweed calcium, CPC, sodium benzoate, propylene glycol, *Krameria* root extract, calcium citrate malate, CI 42090, CI 19140	Elken Sdn Bhd., Malaysia
Watsons herbal mouthwash	Aqua, glycerin, polysorbate 20, poloxamer 407, flavor, *Mentha piperita* oil, CPC, sodium fluoride, propylene glycol, *Mentha piperita* (peppermint) leaf extract, *Thymus vulgaris* (thyme) flower/leaf extract, *Commiphora myrrha* resin extract, *Camellia sinensis* leaf extract, *Aloe barbadensis* leaf extract, citric acid, CI 47005, CI 42090	Watsons Sdn Bhd., Malaysia
Oradex antibacterial mouthwash	Chlorhexidine gluconate (0.12%)	Cavico Sdn Bhd., Malaysia

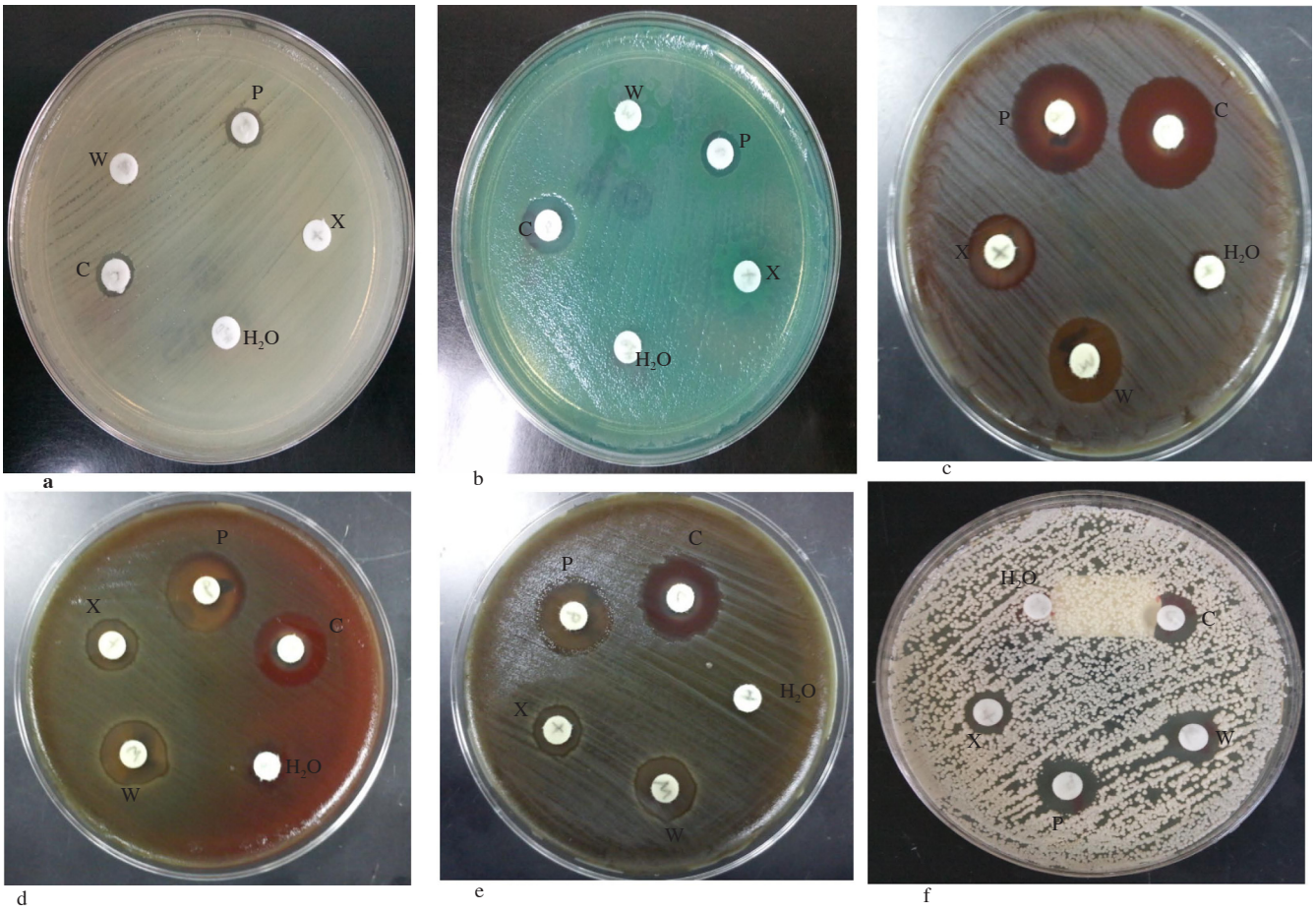

Figure 1. Zones of inhibition produced by the mouthrinses against six tested microorganisms and control in disk diffusion test.
a: *S. aureus*; b: *P. aeruginosa*; c: *S. mutans*; d: *L. salivarius*; e: *S. sobrinus*; f: *C. albicans*; W: Watsons; P: Pesona; X: OX; C: Chlorhexidine-positive control; H_2O: sterile distilled water-negative control.

Table 2
Diameter of inhibition zone (mm) formed by the mouthwashes in the agar-disk diffusion test against six oral microorganisms tested.

Tested organisms	OX	Pesona	Watsons	Oradex (0.12%) chlorhexidine gluconate (positive control)	Sterile distilled water (negative control)	*P* value
S. sobrinus	12.00 ± 0.82	17.00 ± 0.00	15.30 ± 0.47	19.70 ± 0.94	NA	0.250
S. mutans	13.70 ± 0.47	23.30 ± 0.47	17.30 ± 1.25	22.70 ± 1.70	NA	
L. salivarius	13.00 ± 0.00	17.70 ± 1.89	16.30 ± 0.47	19.30 ± 0.94	NA	
S. aureus	NA	12.00 ± 0.00	NA	10.00 ± 0.00	NA	
P. aeruginosa	NA	12.00 ± 0.41	NA	14.00 ± 0.41	NA	
C. albicans	20.00 ± 0.41	21.00 ± 0.41	21.00 ± 0.71	21.00 ± 0.47	NA	

Data are expressed as mean ± SD. NA: No activity.

2.4. Statistical analysis

Results were expressed as mean ± SD. Statistical analysis was done using a statistical package, SPSS windows version 20.0 by applying mean values using non-parametric Kruskal-Wallis test followed by Mann-Whitney U test to determine if there was a significant difference among different formulations of herbal-based mouthrinses. A *P* value of less than 0.05 was considered significant.

3. Results

The zones of inhibition produced by the mouthrinses against tested microorganisms are shown in Figure 1. The mean values and standard deviations of the microbial inhibition zones are shown in Table 2. All herbal-based mouthrinse formulations and Oradex were effective in inhibiting the growth of *S. mutans*, *S. sobrinus*, *L. salivarius* and *C. albicans*. The results showed that all herbal-based mouthrinse formulations had maximum zones of inhibition against *C. albicans*, compared to other microorganisms. Both Watsons and OX mouthrinses did not display any inhibitory effect against *S. aureus* and *P. aeruginosa*. Among the tested mouthrinses, Pesona was the only effective mouthrinse against *S. aureus* and *P. aeruginosa* ($P < 0.05$), similar to Oradex mouthrinse. Pesona mouthrinse formulation appears to be as effective as a non-herbal mouthwash, Oradex to kill *S. aureus* and *P. aeruginosa*. However, statistical analysis showed that no significant difference was observed between tested mouthrinses ($P > 0.05$).

4. Discussion

Maintenance of good oral hygiene is the key for prevention of oral diseases. The principal ingredients of the mouthrinses used in this study may possess many medicinal properties. However, data pertaining to the substantivity of these products are sparse. This study was primarily conducted to evaluate *in vitro* effect of different formulations of herbal-based mouthrinses as a potential agent in the inhibition of *S. sobrinus*, *S. mutans*, *L. salivarius*, *S. aureus*, *P. aeruginosa* and *C. albicans* and to evaluate their effectiveness as compared to Oradex antibacterial mouthwash containing 0.12% chlorhexidine gluconate. Chlorhexidine formulations are considered to be the gold standard anti-plaque and anti-gingivitis mouthrinses due to their prolonged broad spectrum antimicrobial activity and plaque inhibitory potential[11,12].

The results of the present study demonstrated that various herbal-based mouthrinses exhibited wide variations in their effectiveness against the six test microorganisms. All mouthrinses tested exhibited antimicrobial and antifungal effects against at least four microorganisms tested, as exhibited by agar-disk diffusion method. It was probably due to the different formulations of their antimicrobial active ingredients. It was assumed that the active product concentration and its interactions with other substances, in addition to differences in the formulations, would be responsible for different effects[13].

Most of the herbal ingredients in these mouthrinses formulation contained essential oils such as peppermint oil, tea tree oil and eucalyptus oil derived from different types of plants. The herbal extracts of essential oil have been shown to exhibit broad spectrum antimicrobial properties[14-26]. The presence of essential oil of *Melaleuca alternifolia* (tea tree oil) has been shown to exhibit significant inhibitory effect against *Escherichia coli*, *S. aureus*, *Enterococcus faecalis* and *C. albicans*[14-18]. In addition, previous studies have shown that eucalyptus oil exhibited antibacterial property against *S. mutans*[21-23]. All formulations consisted of peppermint oil, which has been proven to exhibit the highest antibacterial activity compared to other different forms of peppermint[24-26].

All herbal-based mouthrinses contain CPC, a broad spectrum antimicrobial agent in its formulation. Mouthrinses containing 0.05% CPC formulated with or without alcohol have been shown to demonstrate broad spectrum antimicrobial activity against both laboratory strains and supragingival plaque bacteria compared to a control mouthrinse without CPC[27]. In the present study, all mouthrinse formulations were effective in inhibiting the growth of *S. mutans*, *S. sobrinus*, *L. salivarius* and *C. albicans*. Among the tested mouthrinses, Pesona was the only effective mouthrinse against *S. aureus* and *P. aeruginosa*, similar to Oradex mouthrinse. Both OX and Watsons mouthrinses did not show any inhibitory action against these two microorganisms. Pesona mouthrinse formulation appears to be as effective as Oradex mouthrinse formulation to kill *S. aureus* and *P. aeruginosa*. The highest antimicrobial activity of Pesona, was most probably due to the synergistic effect between active ingredients of this mouthrinse formulation. The presence of eucalyptus and peppermint oils in its formulation which has also been known for its antibacterial and antifungal effect, may act in synergism with tea tree oil[28]. The mouthrinses formulation containing more than one antimicrobial agent had higher activity against microorganisms.

The data showed the herbal-based mouthrinses formulation exhibited variations in their antimicrobial effectiveness against tested microorganisms. In view of the limitations of *in vitro* studies, it is worth mentioning that these results may not correspond to the actual behaviors of tinctures *in vivo* because they are not exposed to the same conditions found in the oral cavity. Nevertheless, *in vitro* laboratory studies are needed to support the performance of further clinical investigations. The result obtained in this study can serve as a guide for selecting a kind of herbal mouthrinses as well as providing information to the dental professionals about the efficacy of these products.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

The awareness of the public for the need of oral hygiene and healthier life has resulted in an increase in sales and number of brands coming onto the market. Therefore, it is important to assess the effectivity of all available commercial herbal-based mouthrinses.

Research frontiers

The present research work presents antimicrobial activity of three mouthrinses (OX, Pesona and Watsons) by *in vitro* study against six organisms involved in oral diseases. Oradex was used as control.

Related reports

The folklore dentistry has evidence of antimicrobial effectiveness of herbal extracts. All formulations were effective in inhibiting the growth of *S. mutans*, *S. sobrinus*, *L. salivarius* and *C. albicans in vitro*. However, Pesona and Oradex (control) were the only that also inhibit the *S. aureus* and *P. aeruginosa*.

Innovations and breakthroughs

Herbal extracts have being added into novel antimicrobial formulations. In the present study, authors have demonstrated the antimicrobial effectivity of all tested formulations, depending of the

evaluated strain.

Applications

Natural antimicrobial agents have being used for the control and treatment of dental caries, periodontal diseases or fungal diseases in humans. This scientific study reports the effectivity of novel mouthrinses against some pathogenic species, and of the Peasona similar to the chlorhexidine, which is actually the most effective antimicrobial agent.

Peer review

This is a valuable research work in which authors have demonstrated the antimicrobial activity of novel mouthwashes. The activity was assessed by the disk diffusion method.

References

[1] Gamboa F, Estupinan M, Galindo A. Presence of *Streptococcus mutans* in saliva and its relationship with dental caries: antimicrobial susceptibility of the isolates. *Univ Sci* 2004; **9**(2): 23-7.

[2] Carmona LE, Reyes N, González F. Polymerase chain reaction for detection of *Streptococcus mutans* and *Streptococcus sobrinus* in dental plaque of children from Cartagena, Colombia. *Colomb Med* 2011; **42**: 430-7.

[3] Okada M, Kawamura M, Oda Y, Yasuda R, Kojima T, Kurihara H. Caries prevalence associated with *Streptococcus mutans* and *Streptococcus sobrinus* in Japanese schoolchildren. *Int J Paediatr Dent* 2012; **22**(5): 342-8.

[4] Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. *J Clin Microbiol* 2008; **46**: 1407-17.

[5] Belda-Ferre P, Alcaraz LD, Cabrera-Rubio R, Romero H, Simón-Soro A, Pignatelli M, et al. The oral metagenome in health and disease. *ISME J* 2012; **6**: 46-56.

[6] Oluremi BB, Osungunna MO, Idowu OA, Adebolu OO. Evaluation of anticaries activity of selected mouthwash marketed in Nigeria. *Trop J Pharm Res* 2010; **9**(6): 581-6.

[7] Pan PC, Harper S, Ricci-Nittel D, Lux R, Shi W. *In-vitro* evidence for efficacy of antimicrobial mouthrinses. *J Dent* 2010; **38**: S16-20.

[8] Malic S, Emanuel C, Lewis MAO, Williams DW. Antimicrobial activity of novel mouthrinses against planktonic cells and biofilms of pathogenic microorganisms. *Microbiol Discov* 2013; doi: 10.7243/2052-6180-1-11.

[9] Prasanth M. Antimicrobial efficacy of different toothpastes and mouthrinses: an *in vitro* study. *Dent Res J (Isfahan)* 2011; **8**(2): 85-94.

[10] Aneja KR, Joshi R, Sharma C. The antimicrobial potential of ten often used mouthwashes against four dental caries pathogens. *Jundishapur J Microbiol* 2010; **3**(1): 15-27.

[11] Balagopal S, Arjunkumar R. Chlorhexidine: the gold standard antiplaque agent. *J Pharm Sci Res* 2013; **5**(12): 270-4.

[12] Mathur S, Mathur T, Shrivastava R, Khatri R. Chlorhexidine: the gold standard in chemical plaque control. *Natl J Physiol Pharm Pharmacol* 2011; **1**(2): 45-50.

[13] Tanomaru JM, Nascimento AP, Watanabe E, Matoba-Júnior F, Tanomaru-Filho M, Ito IY. Antibacterial activity of four mouthrinses containing triclosan against salivary *Staphylococcus aureus*. *Braz J Microbiol* 2008; **39**: 569-72.

[14] Sailer R, Berger T, Reichling J, Harkenthal M. Pharmaceutical and medicinal aspects of Australian tea tree oil. *Phytomedicine* 1998; **5**(6): 489-95.

[15] Cox SD, Mann CM, Markham JL, Bell HC, Gustafson JE, Warmington JR, et al. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *J Appl Microbiol* 2000; **88**: 170-5.

[16] Santamaria M Jr, Petermann KD, Scudeler SA, Degan V, Lucato A, Franzini CM. Antimicrobial effect of *Melaleuca alternifolia* dental gel in orthodontic patients. *Am J Orthod Dentofacial Orthop* 2014; **145**(2): 198-202.

[17] Thomsen PS, Jensen TM, Hammer KA, Carson CF, Mølgaard P, Riley TV. Survey of the antimicrobial activity of commercially available Australian tea tree (*Melaleuca alternifolia*) essential oil products *in vitro*.*J Altern Complement Med* 2011; **17**(9): 835-41.

[18] Markham JL. Biological activity of tea tree oil. In: Southwell I, Lowe R, editors. *Tea tree: the genus Melaleuca*. Amsterdam: Harwood Academic Publishers; 1999, p. 169-90.

[19] Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG. Composition and antimicrobial activity of essential oils from aromatic plant used in Brazil. *Braz J Microbiol* 2004; **35**(4): 1517-20.

[20] Upadhyay RK, Dwivedi P, Ahmad S. Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. *Asian J Med Sci* 2010; **2**(3): 152-8.

[21] Chaudhari LK, Jawale BA, Sharma S, Sharma H, Kumar CD, Kulkarni PA. Antimicrobial activity of commercially available essential oils against *Streptococcus mutans*. *J Contemp Dent Pract* 2012; **13**(1): 71-4.

[22] Goyal R, Ananad MK. Antibacterial effect of lemongrass oil on oral microorganisms: an *in vitro* study. *J Pharm Sci Innov* 2013; **2**(2): 41-3.

[23] Koteswara RP, Bobbarala V, Aryamithra D, Devi PS, Rao TR. *In vitro* antibacterial activities of plant essential oils against oral bacteria. *Indian J Multi Res* 2008; **4**(4): 507-16.

[24] Singh R, Shushni MAM, Belkheir A. Antibacterial and antioxidant activities of *Mentha piperita* L. *Arabian J Chem* 2011; doi:10.1016/j.arabjc.2011.01.019.

[25] Tyagi AK, Malik A. Antimicrobial potential and chemical composition of *Mentha piperita* oil in liquid and vapour phase against food spoiling microorganisms. *Food Control* 2011; **22**(11): 1707-14.

[26] Saeed S, Naim A, Tariq P. *In vitro* antibacterial activity of peppermint. *Pak J Bot* 2006; **38**(3): 869-72.

[27] Sreenivasan PK, Haraszthy VI, Zambon JJ. Antimicrobial efficacy of 0.05% cetylpyridinium chloride mouthrinses. *Lett Appl Microbiol* 2013; **56**(1): 14-20.

[28] Edris AE, Farrag ES. Antifungal activity of peppermint and sweet basil essential oils and their major aroma constituents on some plant pathogenic fungi from the vapor phase. *Nahrung* 2003; **47**: 117-21.