European Journal of Medicinal Plants
15(4): 1-8, 2016, Article no.EJMP.27095
ISSN: 2231-0894, NLM ID: 101583475

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of the Anti-*Streptococcus mutans* Potential of *Petroselinum crispum*, an *in vitro* Study

Jannan Ghapanchi[1], Abdollah Bazargani[2], Alireza Zariean[3], Mostafa Rezaee[1*], Zahra Ranjbar[1], Arash Karami[4] and Aisa Zamani[3]

[1]Department of Oral Medicine, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Department of Bacteriology and Virology, Shiraz University of Medical Sciences, Shiraz, Iran.
[3]School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.
[4]Department of Oral and Maxillofacial Surgery, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Authors JG, AB and Alireza Zariean designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MR, Aisa Zamani, ZR and AK managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2016/27095
Editor(s):
(1) Paola Angelini, Department of Applied Biology, University of Perugia, Italy.
(2) Elena Maria Varoni,Dipartimento di Scienze Biomediche, Chirurgiche ed Odontoiatriche, University of Milan, Italy.
(3) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Anonymous, University Estadual Paulista (Unesp), Brazil.
(2) Pedro Henrique Sette de Souza, Universidade de Pernambuco, Brazil.
(3) Patrícia Diogo Nunes, University of Coimbra, Portugal.
(4) Anonymous , University of Gothenburg, Sweden.
Complete Peer review History: http://www.sciencedomain.org/review-history/15514

Original Research Article

Received 18th May 2016
Accepted 13th July 2016
Published 26th July 2016

ABSTRACT

Background and Aim: The use of medicinal plants and traditional remedies has become increasingly widespread. Parsley is one of the traditional herbs used for treatment and prevention of various illnesses. This study aims to assess the *in vitro* antimicrobial effect of *Petroselinum crispum* (parsley) on *Streptococcus mutans* and compare it to antibacterial activity of different concentrations of chlorhexidine 0.2%.
Materials and Methods: The minimal bactericidal concentration (MBC) and minimal inhibitory

**Corresponding author: E-mail: ghabanj@sums.ac.ir;*

concentration effects of parsley on *S. mutans* isolates were investigated by Agar well diffusion and broth microdilution assays.
Results: *S. mutans* was treated with the extract in various concentrations according to the CLSI's protocol. The median minimal inhibitory concentration (MIC) of ethanolic extract exhibited a bactericidal effect on *S. mutans strains in* 2500 ppm comparing to aqueous extract that showed bactericidal activity in 10000 ppm, p=0.001. CHX demonstrated anti streptococcal activity in 260 ppm concentration revealing more intensive bactericidal effect than parsley, p=0.001. This research showed that Chlorhexidine significantly has higher anti *S. mutans activity than parsley.*
Conclusion: The results indicate that parsley (*Petroselinum crispum*) has a lower *anti S. mutans* activity *in vitro*. Its bacteriostatic activity on *S. mutans* strains is less than that of chlorhexidine mouth wash p=001. The use of parsley as a preventive measure for dental caries needs more investigations.

Keywords: Bactericidal effect; parsley; Streptococcus mutans.

1. INTRODUCTION

Parsley is a species of *Petroselinum* in the family *Apiaceae*, native to the central Mediterranean region (Southern Italy, Algeria, and Tunisia), naturalized elsewhere in Europe, and widely cultivated as an herb, a spice, and a vegetable.

The word "parsley" is a merger of the Old English *petersilie* (which is identical to the contemporary German word for *parsley*: *Petersilie*) and the Old French *peresil*, both derived from Medieval Latin *petrosilium*, from Latin *petroselinum* [1].

Streptococcus mutans (*S. mutans*) is facultative anaerobic coccus-shaped, Gram-positive bacteria commonly found in the oral cavity and play a major role in tooth decay formation and in metabolizing sucrose to lactic acid.

There are twenty-five species of oral streptococci in human oral cavity. Each species develops specialized properties for colonization in oral sites and constantly changes its conditions to fight with competing bacteria. Imbalances in the microbial flora can initiate oral diseases. Under specific conditions, commensal streptococci can be changed into opportunistic pathogens, which can cause disease and do damage to the host. Therefore, oral streptococci may be harmless or harmful bacteria according to oral environmental condition [2,3].

Herbal medicine has become increasingly widespread and has been enriched by the vast biodiversity and by the combining indigenous, African, and European cultures [4].

The antimicrobial properties of plant extracts and isolated compounds have been studied by a number of investigators worldwide [5,6]. In Brazil, herbal medicine is increasing at a rate of 20% a year, following the re-evaluation of the global use of medicinal plants for the treatment of several diseases [7]. Many researchers have focused on determining the anti-viral, bacterial, fungal and antioxidant effects of vegetables.

Antioxidant capacity of the essential oil extracted from parsley (*Petroselinum crispum)* has been evaluated by three different *in vitro* assays in China: b-carotene bleaching assay, DPPH_ free radical scavenging assay and Fe2+-metal chelating assay. Results indicate that while parsley oil has a certain degree of antioxidant activities regarding b-carotene bleaching and free radical scavenging activities, its metal chelating capacity is not significant [8].

Another study performed in assessed the antibacterial capacity of crude hydroalcoholic extracts, fractions, and compounds of *Rosmarinus officinalis* and *Petroselinum crispum* against the bacteria causing urinary tract infection.

The result showed that crude extract obtained from the leaves and stems of P. *crispum* furnished MIC and MBC > 400 _g/mL for all the tested bacterial strains, except for *P. aeruginosa* (ATCC 14502). The hydroalcoholic extract of *R. officinalis* manifested *in vitro* activity against Gram-positive bacteria, with satisfactory MBC for the clinical isolate *S. saprophyticus.* Fractions and pure compound rosmarinic acid did not show promising results for Gram-negative bacteria [9].

An investigation was designed in Saudi Arabia to study the anti-cancer activity of seed extracts of *Portulaca oleracea* (PO) and *Petroselinum sativum* (PS on the human hepatocellular

carcinoma cells (HepG2). The results showed that PO and PS extracts significantly reduced the cell viability of HepG2 depending on their concentrations. The study also showed that PO and PS exposed cells decreased the normal morphology and adhesion capacity of HepG2 cells. HepG2 cells exposed to 50 µg/ml or higher concentrations of PO and PS lost their typical morphology, became smaller in size, and appeared in rounded bodies. In general, this research revealed preliminary screening of anti-cancer activity of *Portulaca oleracea* and *Petroselinum sativum* extracts against HepG2 cells, which can be further employed for the development of a potential therapeutic anticancer agent [10].

Many other studies have been performed in order to measure anti streptococcus efficacy of some foods and herbs. Ghabanchi et al. [11] assessed the *in vitro* antimicrobial effect of honey on *Streptococcus mutans* in Shiraz, Iran. They found that honey (*Apis mellifera*) has bacteriostatic activity when tested *in vitro*.

Another study investigated the inhibitory effect of the crude extracts of six herbs on adherence of *Streptococcus mutans* (*S. mutans*) ATCC 25175 and TPF-1 *in vitro*. *Andrographis paniculata*, *Cassia alata*, Chinese black tea (*Camellia sinensis*), guava (*Psidium guajava*), *Harrisonia perforate,* and *Streblus asper* were extracted with 50% or 95% ethanol and dried. The result demonstrated that all extracts, except *Streblus asper*, showed significant inhibitory effect on bacterial adherence to glass surfaces [12].

A study in Korea evaluated and compared the antibacterial activity of herbal extracts against normal oral streptococci, planktonic and biofilm of *S. mutans*. The result indicated that extract of *S. flavescens* might be a potential candidate for prevention and management of dental caries [13].

Streptococcus mutans is associated with dental caries. A cariogenic biofilm, in particular, has been extensively studied for its role in the formation of dental caries. The control of this microorganism growth prevents caries development [13]. Parsley is commonly used in different Iranian foods and has antibacterial activity. Therefore, the present study has been conducted in Shiraz, Iran to evaluate the anti *S Mutans* activity of parsley extract and compare it to chlorhexidine anti-bacterial activity.

2. MATERIALS AND METHODS

Chlorhexidine 0.2% mouth rinse, produced in Iran under the commercial name of Behsa, was used. Lyophilized *Streptococcus mutans* ATCC 35668 (PTCC 1683) was obtained from the Persian Type Culture Collection (PTCC), Iran. Parsley was collected from Shiraz agriculture school.

2.1 Preparation of Herbal Extracts

300 ml of sterilized distilled water was added to 30 g of ground dried plant, heated below the boiling point and stirred for 2 ½ - 3 hours. The extract was filtered by muslin cloth, then by filter paper (Whatman No. 1) and subsequently stored in the refrigerator at 5ºC for consumption [14]. The alcoholic extract prepared in the pharmacy school.

2.2 Cultivation the Reference Strain

Lyophilized reference *S. mutans* was poured into Triptycase Soy Broth (TSB) medium and was incubated in an appropriate atmosphere (H2:CO2:N2 10:10:80) at 37℃ for 24 hours. Cultivated bacteria were then streaked onto the blood agar media. The media were incubated for 24 hours at 37℃. A well-isolated colony of the reference strain was picked from the agar plate and was aseptically transferred into the 5 ml of sterile nutrient broth medium. The broth medium was then incubated in anaerobic atmosphere (H2:CO2:N2 10:10:80) at 37℃ for 24 hours.

For antimicrobial susceptibility testing, the turbidity of bacterial suspension was adjusted to a 0.5 McFarland standard and it is comparable to a bacterial suspension of 1.5×10^8 CFU/ml [15].

2.3 Broth Microdilution Assay

Broth microdilution test was performed in 96-well plates. 200 µl of the herb used with the highest concentrations was added into the first column wells and 100 µl of nutrient broth was added into the remaining wells. Then, the two-fold serial dilution of medicinal herb solution was made by drawing up 100 µl of medicinal herb solution from the first column wells into the second column and then move it onto the next, column to achieve the final concentration.

Next the 0.5 McFarland bacterial suspension (1.5 x 10^8 CFU/mL) [15] was diluted to a 10% suspension to yield 10^7 CFU/mL. 5 µl of this suspension was inoculated into each well of

microliter plates to obtain the final concentration of bacteria approximately 5 x 10^4 CFU/well (10^5 CFU/ml). The last two wells were positive and negative controls, respectively. The positive control was inoculated just with bacterial suspension, while the negative control well was left without inoculation. The 96-micro well plates were sealed using a perforated plate seal and incubated in an appropriate atmosphere (H2:CO2:N2 10:10:80) at 37℃ for 48 hours. The MIC values were recorded as the lowest concentrations where no viability was observed in the wells of 96-micro well plates after the incubation period.

For comparison, the same procedure was performed for chlorhexidine as the popular effective mouth wash and each test was done in triplicate. To determine the minimal bactericidal concentration (MBC) of the tested agents, 10 µl of the broth medium in each well that had not shown any growth was collected and inoculated on a fresh blood agar medium. Another blood agar medium was also inoculated with S*. mutans* as a control. The plates were incubated at 37℃ for 24 hours. The concentration at which no growth was detectable was considered as MBC.

2.4 Statistical Analysis

Statistical analysis was done by SPSS version 20.0 (IBM SPSS, IBM corporation, Chicago, IL, USA) using Mann -Whitney test to compare the groups.

3. RESULTS

S mutans was treated with the extract in various concentrations according to the CLSI's protocol [16]. The susceptibility test of *S. mutans* for parsley extract was investigated. The median minimal inhibitory concentration (MIC) of ethanolic extract exhibited a bactericidal effect on *S. mutans strains in* 2500 ppm comparing to aqueous extract that showed bactericidal activity in 10000 ppm, p=0.001.

The result also indicates that there is a significant difference between ethanolic and aqueous parsley extracts and chlorhexidine (CHX) mouth wash. CHX demonstrated anti streptococcal activity in 260 ppm concentration revealing more intensive bactericidal effect than parsley, p=0.001.

The median minimal bactericidal concentration (MBC) of ethanolic extract exhibited a bactericidal effect on *S. mutans strains in* 7500 ppm comparing to aqueous extract that exhibited bactericidal activity in 15000 ppm, p=0.001. Ethanol extract in comparison with the aqueous extract is more effective and has a greater deterrent CHX showed anti S *mutans* activity in 520 ppm concentration that was more efficient than both alcoholic and aqueous extracts, p=0.001.

The median inhibitory zone around the disc impregnated with parsley ethanolic extract was 20.7 mm and that of aqueous extracts was 10.8 mm, p=0.005.

The median inhibitory zone around the disc impregnated with CHX (disk diameter test) was 60.7 mm demonstrating higher anti *S. mutans* activity than both alcoholic and non-alcoholic *Petroselinum crispum* extracts, p=0.001.

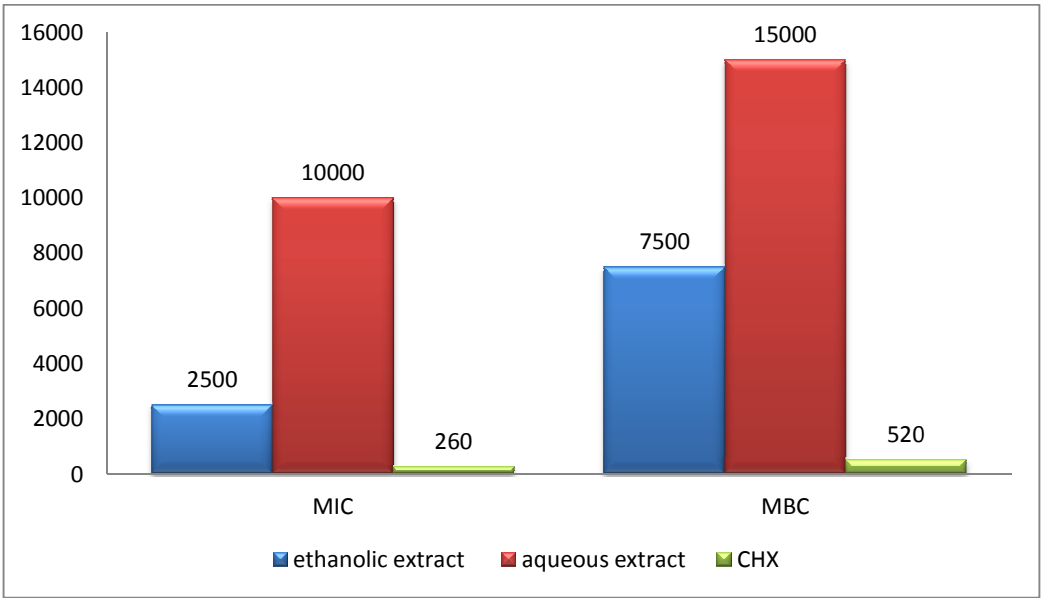
The medians of inhibition zone (well cut into the agar) around alcoholic and aqueous extracts were 14.95 mm and 12 mm, respectively, p=007.

A significant result has been found with different concentrations of chlorhexidine comparing with both parsley extracts. The median of inhibition zone was 34.8 mm. p=0.001.

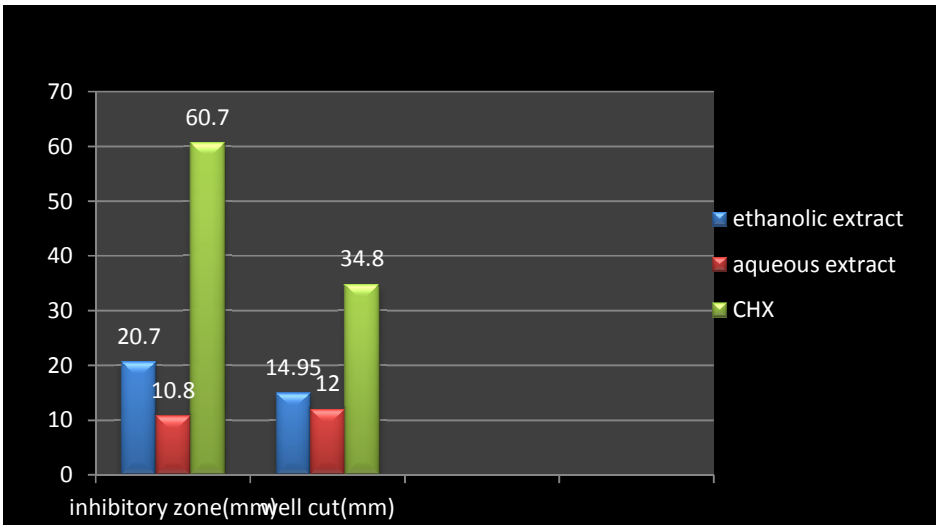
This research showed that Chlorhexidine significantly has higher anti *S. mutans* activity than parsley.

Table 1. The median minimal inhibitory concentration (MIC) and the median minimal bactericidal concentration (MBC) of bactericidal effect and the median inhibitory zone around disc impregnated and well cut into the agar of parsley extracts and chlorhexidine against *S. mutans strains*

	Ethanolic extract of parsley	Aqueous extract of parsley	P value	Chlorhexidine	P value
MIC	2500 ppm	10000 ppm	0.001	260 ppm	0.001
MBC	7500 ppm	15000 ppm	0.001	520 ppm	0.001
Inhibitory zone	20.7 mm	10.8 mm	0.005	60.7 mm	0.001
Well cut	14.95 mm	12 mm	0.007	34.8 mm	0.001



Graph 1. The median minimal inhibitory concentration (MIC) and the median minimal bactericidal concentration (MBC) of bactericidal effect of parsley extracts and chlorhexidine against *S. mutans strains*



Graph 2. The median inhibitory zone around disc impregnated and well cut into the agar of parsley extracts and chlorhexidine against *S. mutans strains*

4. DISCUSSION

Natural products have recently been demonstrated as an alternative to synthetic substances for prevention of tooth decay. Many studies have reported that antibacterial activity investigation is based on folk medicine; that is, the selection of plants that are commonly used by various populations for medical treatments.

The antibacterial properties of parsley against medically important bacteria have been well documented [8,9] but this information is not completely available for the oral bacteria and specifically for oral *Streptococci*.

On the basis of MIC determination, Ojala et al. [17] reported the antimicrobial activity of the methanol extract of the plant species *P. crispum*,

containing coumarins, against the clinical isolates *Bacillus subtilis*, *P. aeruginosa*, *S. epidermidis*, *S. aureus* and *Saccharomyces cerevisiae*. According to these authors, a moderate antimicrobial activity, with MIC ranging from 200 _g/mL to 350 _g/mL, was achieved. Using the hydroalcoholic of the same plant, our group obtained the same MIC values against the standard strain of *P. aeruginosa.*

Silva et al. [18] investigated the action of the hydroalcoholic extract of *R. officinalis* on *Streptococcus mitis* (ATCC 9811), *Streptococcus sanguinis* (ATCC 10556), *Streptococcus mutans* (ATCC 25175) and *Streptococcus sobrinus* (ATCC 27609) which are the predominant bacterial species in the supragingival biofilm. *Lactobacillus casei* (ATCC 7469) obtained by the MIC technique. The MIC values lay between 40 and 120 g/mL, except for *S. mitis* (ATCC 9811) which yielded MIC value greater than 400 g/mL. According to these authors, the rosemary extract was effective against the tested strains whereas in our study the selected bacterial strains did not give evidence of such promising results in both extracts .In particular, *S. mutans* biofilm rather than its planktonic counterpart is directly associated with dental caries formation [19].

S. mutans synthesize glucan as exopolysaccharide from sucrose by the use of glucosyltransferases [20]. Therefore, cariogenic biofilm which is an oral biofilm containing *S. mutans* consists of oral bacteria, exopolysaccharide, bacterial debris, etc. [21].

Kim et al. [22] found that *sophoraflavanone G,* a fraction of *Sophora flavescent* extract, has a bactericidal effect on *S. mutans* and *S. sobrinus* at a concentration up to 100 µg/ml which is not compatible with the results of our study. An appropriate antimicrobial agent for clinical application should just target oral pathogens without disturbing normal oral streptococci [23]. Thus, susceptibility assay of normal oral streptococci for the herbal extracts was investigated.

Alizadeh Behbahani, et al. [24] reported that ethanol extract in comparison with the aqueous extract is more effective and has a greater deterrent. It may be due to extracting more effective materials from Avicennia marina by ethanol. These findings are consistent with the results obtained in this study. The results of their study showed that the ethanol extract of parsley exhibited stronger antibacterial activity against *S. mutans* compared with that of aqueous extract.

We used Chlorhexidine 0.2% in different concentrations as our controls. A significant inhibition zone was observed in many isolates in agar well diffusion assay by the concentrations of 30-80% in broth of microdilution assay. It seems that the content of parsley has not a significant inhibitory effect on *S. mutans.* Further studies on anti *S. mutans* effect of *Petroselinum crispum*, especially in clinical trials, are required to determine whether it can be used as a preventive measure for dental caries or not .

Haas et al. [25] reported that Chlorhexidine showed good results in reducing plaque and gingivitis. However, because it has more adverse effects after continuous use, it should not be indicated for long-term periods.

Varoni et al. [26] mentioned that chlorhexidine (CHX) is one of the most commonly prescribed antiseptic agents in the dental field. It has a long-lasting antibacterial activity with a broad-spectrum of action and it has been shown to reduce plaque, gingival inflammation and bleeding. Its use is considered a powerful adjuvant to mechanical oral hygiene (brushing and flossing), especially in those cases in which it cannot be performed correctly. Data support its periodic use as an adjuvant to normal brushing and flossing in subjects unable to maintain proper oral hygiene due to physical and/or mental impairment, or lack of motivation, or decreased salivary rate. CHX is also a useful alternative to mechanical oral hygiene procedures in those cases in which they are contraindicated, e.g. after a surgical procedure, or as a preoperative rinse before procedures in which use of a dental dam is not possible.

5. CONCLUSION

Parsley (*Petroselinum crispum*) extracts showed lower in vitro bacteriostatic activity on *S mutans* strains than chlorhexidine mouth wash. Further studies are suggested to determine its preventive measure for dental caries.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The authors thank the vice chancellor of Shiraz University of Medical Sciences for supporting this research (Grant≠8792). The assistance of the Editor-in-Chief and Journal Manager is also appreciated. This article is based on the thesis #1746 by Alireza Zareian, dentistry student at Shiraz University of Medical Sciences.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Lewis C, Short C. A Latin Dictionary, on Perseus Digital Library.
2. Loesche WJ. Microbiology of dental decay and periodontal disease. In: Baron S, editor. Medical microbiology. Galveston TX: The University of Texas Medical Branch at Galveston; 1996.
3. Nicolas GG, Lavoie MC. *Streptococcus mutans* and oral streptococci in dentalplaque. Canadian Journal of Microbiology. 2011;57(1):1-20.
4. Oliveira CF, Junqueira RG, Stehmann JR, Brandão MGL. Potential of medicinal plants as a source of new antimalarialdrugs: Species listed in the bibliography Ethno Brazil. Braz J Med Biol Res. 2003;5:23-31.
5. More G, Tshikalange TE, Lall N, Botha F, Meyer JJ. Antimicrobial activity of medicinal plants against oral microorganisms. J Ethnopharmacol. 2008; 119:473-477.
6. Al-Bakri AG, Othman G, Afifi FU. Determination of the antibiofilm, antiadhesive, and anti-MRSA activities of seven *Salvia* species. Pharmacogn Mag. 2010;6:264-270.
7. Cartaxo SL, Souza MM, De Albuquerque UP. Medicinal plants with bioprospecting potential used in semi-arid Northeastern Brazil. J Ethnopharmacol. 2010;131:326-342.
8. Hui Zhang AB, Feng Chen B, Xi Wang C, Hui-Yuan Yao. Evaluation of antioxidant activity of parsley (*Petroselinum crispum*) essential oil and identification of its antioxidant constituents. Food Research International. 2006;39:833–839.
9. Petrolini FV, Lucarini R, de Souza MG, Pires RH, Cunha WR, Martins CH. Evaluation of the antibacterial potential of *Petroselinum crispum* and *Rosmarinus officinalis* against bacteria that cause urinary tract infections. Braz J Microbiol. 2013;44(3):829-34.
10. Farshori NN, Al-Sheddi ES, Al-Oqail MM, Musarrat J, Al-Khedhairy AA, Siddiqui MA. Cytotoxicity assessments of *Portulaca oleracea* and *Petroselinum sativum* seed extracts on human hepatocellular carcinoma cells (HepG2). Asian Pac J Cancer Prev. 2014;15(16):6633-8.
11. Ghabanchi J, Bazargani A , Daghigh Afkar M, Balady Foroshan S, Dad Ayeen S. *In vitro* assessment of anti-*Streptococcus mutans* potential of honey. IRCMJ. 2010; 12(1):61-64.
12. Limsong J, Benjavongkulchai E, Kuvatanasuchati J. Inhibitory effect of some herbal extracts on adherence of *Streptococcus mutans*. J Ethnopharmacol. 2004;92(2-3):281-9.
13. Lee SH. Antimicrobial effects of herbal extracts on *Streptococcus mutans* and normal oral streptococci. J Microbiol. 2013; 51(4):484-9.
14. Al-Neemy U, AL-Jebury SH. The inhibitory effect of some medicinal plant extracts to *Streptococcus pyogenes*. Duhok Zanko J. 2006;11(1):45-51.
15. Siqueira JF, Araújo MC, Garcia PF, Fraga RC, Dantas CJ. Histological evaluation of the effectiveness of five instrumentation techniques for cleaning the apical third of root canals. J Endod. 1997;23(8):499–502.
16. Baek DH. Screening of antimicrobial activity among the therapeutic herbal extracts on dental pathogens. Int'l J. Oral Biol. 2007;32:75-78.
17. Ojala T, Remes S, Haansuu P, Vuorela H, Hiltunen R, Haahtela K, Vuorela P. Antimicrobial activity of some coumarin containing herbal plants growing in Finland. J Ethopharmacol. 2000;73:299-305.
18. Silva MSA, Silva MAR, Higino JS, Pereira MSV, Carvalho AAT. Atividade antimicrobiana e antiaderente *in vitro* do extrato de *Rosmarinus officinalis* Linn. sobre bactérias orais planctônicas. Rev Bras Farmacogn. 2008;18:236-240.
19. Lee SH, Choi BK, Kim YJ. The cariogenic characters of xylitol-resistant and xylitol-sensitive *Streptococcus mutans* in biofilm

formation with salivary bacteria. Arch. Oral Biol. 2012;57:697–703.
20. Monchois V, Willemot RM, Monsan P. Glucansucrases: Mechanism of action and structure-function relationships. FEMS Microbiol. Rev. 1999;23:131-151.
21. Marsh PD, Bradshaw DJ. Dental plaque as a biofilm. J. Ind. Microbiol. 1995;15:169–175.
22. Kim CS, Park SN, Ahn SJ, Seo YW, Lee YJ, Lim YK, Freire MO, Cho E, Kook JK. Antimicrobial effect of sophoraflavanone G isolated from *Sophora flavescens* against mutans streptococci. Anaerobe. 2013;19:17–21.
23. Sbordone L. Bortolaia C. Oral microbial biofilms and plaque-related diseases: Microbial communities and their role in the shift from oral health to disease. Clin. Oral Investig. 2003;7:181–188.
24. Alizadeh Behbahani B, Tabatabaei Yazdi F, Shahidi F, et al. Antimicrobial activity of *Avicennia marina* extracts ethanol, methanol and glycerin against *Penicillium digitatum* (citrus green mold). Sci J Microbiol. 2012;1(7):147-51.
25. Haas AN, Pannuti CM, Andrade AK, Escobar EC, Almeida ER, Costa FO, Cortelli JR, Cortelli SC, Rode SD, Pedrazzi V, Oppermann RV. Mouthwashes for the control of supragingival biofilm and gingivitis in orthodontic patients: Evidence-based recommendations for clinicians. Braz Oral Res. 2014;28(spe):1-8. Epub 2014 Jul 11.
26. Varoni E, Tarce M, Lodi G, Carrassi A. Chlorhexidine (CHX) in dentistry: State of the art. Minerva stomatol. Review. English, Italian. 2012;61(9):399-419.
PubMed PMID: 22976567.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15514