The Effect of Hydro Alcoholic Extract of Seven Plants on Cariogenic Bacteria- An *in Vitro* Evaluation

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Abstract

Aim: To compare the antibacterial effect of hydro alcoholic extract of *Salvia officinalis, Pimpinella anisum, Satureja hortensis, Rhus coriaria, Carum copticum, Mentha longifolia, Achillea millefolium* against *Streptococcus mutans, Lactobacillus rhamnosus and Actinomyces viscosus* through two *in vitro* methods.

Methods: In this experimental study, hydro-alcoholic extracts have been prepared from the shoot of *Salvia officinalis, Satureja hortensis, Mentha longifolia and Achillea millefolium*, the seed of *Pimpinella anisum and Carum copticum* and the fruit of *Rhus coriaria* with maceration method. Their antibacterial activity against *Streptococcus mutans, Lactobacillus rhamnosus* and *Actinomyces viscosus* have been evaluated with broth macrodilution and agar diffusion methods.

Results: In Broth macrodilution method MIC (Minimum Inhibitory Concentration) of *Pimpinella anisum, Salvia officinalis, Mentha longifolia, Achillea millefolium, Satureja hortensis, Carum copticum* and *Rhus coriaria* for *Streptococcus mutans* were respectively 12.5, 6.25, 12.5, 50, 50, 12.5 and 50 µg/ml, for *Lactobacillus rhamnosus* 12.5, 1.56, 3.12, 12.5, 6.25, 6.25 and 6.25 µg/ml and for *Actinomyces viscosus* 50, 12.5, 100, 50, 100, 25 and 25 µg/ml. In Agar diffusion method *Pimpinella anisum*, *Salvia officinalis* and *Rhus coriaria* against *Streptococcus mutans*, *Pimpinella anisum, Carum copticum* and *Rhus coriaria against Lactobacillus rhamnosus* and *Mentha longifolia, Rhus coriaria* and *Carum copticum* against *Actinomyces viscosus* had antibacterial effects.

Conclusion: All seven extracts had growth inhibitory effects on all three bacteria. *Salvia officinalis* had the greatest inhibitory effect on growth of all three bacteria. All of the extracts except *Carum copticum* had bactericidal effect in the range of concentration. By agar diffusion method *Rhus coriaria* had antibacterial effect against all three cariogenic bacteria.

Key words: Dental caries, Antibacterial agents, Cariogenic bacteria

Introduction

Dental caries is a multi-factorial disease with a microbial nature. Although dental caries is probably one of the most common chronic diseases in the world [1], there has never been any preventive programs to eradicate this disease like programs to fight against polio and small pox.

The destructive effects of this disease are the loss of teeth, pain and esthetic defects. Using antibiotics and steroids against cariogenic bacteria to treat or prevent dental caries may change the oxidation reduction potential of saliva, weaken the activity of lysozyme, facilitate the allergic reactions and reduce the body resistance to pathogenic factors [2]. On the other hand, attraction to traditional medicine and herbal drugs in different fields of medicine has progressed because plants have been used as drugs since a few centuries ago [3]. The use of traditional medicine is one way to make new drugs. Nowadays there are 119 drugs with plant origin that have been brought out of only 90 species from 250000 known species [4]. But it is almost impossible, very expensive and time consuming to search all effective ingredients of all plants against every disease [4]. Therefore one of the best and the most acceptable strategies in the world to discover, use and research about drug plants is to rely on local (native) knowledge.

Taking care of oral and dental health was important in Iran's traditional medicine and there was a chapter about oral and dental disorders in books called "Treatments" [5].

Dental caries highly increases in the presence of acidogenic bacteria like *Streptococcus mutans* [1,6]. *S. mutans* is the first and the most important microorganism in dental plaque and its cariogenicity was proven [1,7,8]. This microorganism plays the main role in initiation of caries lesion formation while *Lactobacillus* contributes to caries progression [7,8] and also causes root caries lesions. In addition to these two microorganisms, *Actinomyces viscosus* plays an important role too [7,8]. Therefore decontamination of bacterial base of caries is one of the ways to remove this widespread infection. Based on this information we have selected seven plants *[Salvia officinalis, Pimpinella anisum, Satureja hortensis, Rhus coriaria, Carum copticum, Menthe longifolia, Achillea millefolium*], which were used to treat oral and dental defects [5,9-13] in Iran's traditional medicine; to research about their antibacterial effects against cariogonic bacteria. Besides, the modern medical texts have also noticed the medical usages of these plants [14,15]. Although there have been some researches about antioxidant and antimicrobial effects of *A. millefolium* [16], *P. anisum* [17,18], *M. longifolia* [19], *S. hortensis* [20,21], *C. copticum* [22], *R. coriaria* [22] and also

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different medical effects of *S. officinalis* [23-29] in recent years, there hasn't been any studies about the antibacterial effect of these plants against cariogenic bacteria except for one research which evaluated the antimicrobial effect of *S. officinalis* only against *S. mutans* [26].

In the present study *S. officinalis* which is more well-known was selected as an herbal positive control. According to the fact that these plants are native in Iran and preparation of their extraction is possible, the goal of this research is to identify the antibacterial effect of seven plants [*S. officinalis, P. anisum, S. hortensis, R. coriaria, C. copticum, M. longifolia, A. millefolium] against Streptococcus mutans, Lactobacillus rhamnosus* and *Actinomyces viscosus* through two experimental methods. The null hypotheses were: 1- All extracts would have antibacterial effect against three cariogenic bacteria. 2- There would be no differences between seven extracts.

Methods

Plant extraction

Extraction was done by "maceration" method. First of all 50 gr of the shoot of *S. officinalis, S. hortensis, M. longifolia* and *A. millefolium,* the seed of *P. anisum* and *C. copticum* and the fruit of *R. coriaria* in the dried form were weighted with a digital balance (LibROR AEU – 210 Japan). After that, they were mashed and then added to 1500cc of a solvent (half ethanol and half water) and were shaken (Heidolph, Unimax 2010 Germany) by 90 cycle/min for 48 hours until they got homogenous. After that the solutions were filtered with strainer (watmann 0/5mm USA) and put on rotary evaporator (Heidolph WD 2000) to evaporate the solvent. Finally the pure extracts were kept in sterile vials in refrigerator to be used in microbial tests.

Antibacterial susceptibility tests

First of all, to prepare the bacteria [*Streptococcus mutans* (ATTC: 35668), *Lactobacillus rhamnosus* (ATCC: 7469), *Actinomyces viscosus* (ATCC: 15987)] the lyophilized samples were cultured in broth medium overnight (24h in 30-35°C). After turbidity occurring in broth medium, the samples were cultured on solid environment (BHI agar (Spain CONDA) for *A. viscosus*, BHI agar (8 CONDA) + 5% blood sheep for *S. mutans*, MRS agar (Germany,Merck) for *L. Rhamnosus*) to ensure about their purity. After that the antibacterial effect of every extract was evaluated against 3 bacterial species by two methods.

Broth macrodilution method

This method was introduced according to CLSI (Clinical Laboratory Standard Institute 2006 A4 USA) protocol. First, 800 µg/ml stock solution of every extract was prepared and sterilized with 0/22 µm Millipore filter. Then the extracts were diluted by serial dilution method in 11 tubes filled by broth medium [Thyioglycollate Medium (Difco, USA) for *A. viscosus*, BHI Broth (CONDA, Spain) for *S. mutans*, MRS Broth (Germany, Merck) for *L. rhamnosus*]. (At the end of dilution there was 500 µl of culture medium and the extract in each tube). Bacterial suspensions were prepared according to 0/5 Macfarnland [30] and then they were diluted with culture medium to the amount of 1/100 to achieve $1/5 \times 10^8$ CFU (colony forming unit) in one milliliter. After that 500 µl of bacterial suspension was added to each tube. Finally the range of concentration of tubes was 0/18 – 200 µg/ml. Incubation was done for 20h in 37°C. Once cultured the tubes were evaluated by turbidimetry. The concentration of the first tube which had no growth was known as MIC (Minimum Inhibitory Concentration) of that extract for bacterial growth. Then the samples from no-growth-tube were cultured in solid agar medium in the plate. The first plate which had no growth was known as MBC (Minimum Bactericidal Concentration) of the extract for that bacterium. Culture mediums with extract, and without bacteria; and culture medium with distilled water and bacteria, were both used as negative control. Culture medium with chlorhexidine and bacteria was positive control. *S. mutans* and *A. viscosus* were incubated in anaerobic jar and beside CO_2. These processes were repeated three times for all seven extracts and three bacteria.

Agar well diffusion Method

This method was introduced according to Bakri & Douglas [31] protocol. First, 800 µg/ml stock solutions were prepared from each extract and sterilized with 0/22 µm Millipore filters. Then dilutions of extracts were done with sterile water in the range of 0/78 – 400 µg/ml. After preparing the suitable agar culture, pouring them to plates of 4mm thickness, and making the bacterial suspension in the amount of $1/5 \times 10^6$ cfu/ml (like the previous method); the bacterial suspension was then spread on the plate surfaces with a swap. Then some wells were punched on agar surfaces with 6mm diameter and 30mm distance between each. After that 500 µl of different dilution of extracts were poured to the wells. Finally they were incubated in 37°C for 20h. Zone of inhibition around the wells were evaluated and measured by millimeter. Distilled water in one well was negative control, and chlorhexidine in another one was positive control.

Statistical analysis was done by Kruskal – Wallis between groups, and if they were significant, each pair group was then compared with another by Dunn – Procedure test (P-value < 0.05).

Results

The results of broth macrodilution method

The MIC and MBC of extracts against 3 under study bacteria are given in *Table 1*.

All extracts inhibited the growth of all bacteria. *S. officinalis* had the highest inhibitory effect against all three bacteria. Beside the bacteriostatic effect of all extracts, they have also had bactericidal effects against the three bacteria in this evaluated range; except *C. copticum* against *Lactobacillus* in which it's MBC was more than 200 µg/ml. [lower amount of MIC and MBC means the higher antibacterial effects].

The results of agar well diffusion

For *S. mutans,* only *S. officinalis, P. anisum* and *R. coriaria* had growth inhibitory effect and the effect of *S. officinalis* and *R. coriaria* were higher and significantly more than *P. anisum* in the concentration of 100, 200 and 400 µg/ml ($P < 0.001$). In the concentration of 25 and 50 µg/ml, *R. coriaria* had the highest antibacterial effect which was significantly higher than *P. anisum* (p=0.002) and *S. officinalis* (P=0.001). Also in lower concentrations there weren't any zone of inhibition (*Figure 1*).

Table 1. *MIC and MBC of the extracts (µg/ml) for the bacteria.*

Antibacterial effect								Statistical analysis*
extrac(µg/ml) Bacterium	***Rhus coriaria***	***Pimpinella anisum***	***Salvia officinalis***	***Mentha longifolia***	***Satureja hortensis***	***Carum copticum***	***Achillea millefolium***	
Lactobacillus rhamnosus (MIC)	6.25	12.5	1.56	3.12	6.25	6.25	12.5	S.a< M.l< S.h, C.c, R.c< A.m, P.a**
Lactobacillus rhamnosus (MBC)	6.25	12.5	12.5	6.25	100	>200	12.5	M.l, Rc< S.a, P.a, A.m< S.h< C.c**
Streptococcus mutans (MIC)	50	12.5	6.25	12.5	50	12.5	50	S.a< P.a, M.l, C.c< A.m, S.h, R.c**
Streptococcus mutans (MBC)	100	200	50	100	200	200	200	S.a< M.l, R.c< C.c, S.h, P.a, A.m**
Actinomyces viscosus (MIC)	25	50	12.5	100	100	25	50	S.a< R.c, C.c< P.a, A.m< S.h, M.l**
Actinomyces viscosus (MBC)	25	100	12.5	100	100	25	100	S.a< R.c, C.c< P.a, A.m, S.h, M.l**

* < means the left one has significantly (p<0.05) lower MIC or MBC than the right ones.
** S.a=Salvia officinalis, P.a= Pimpinella anisum, S.h= Satureja hortensis, R.c=Rhus coriaria, C.c=Carum copticum, M.l= Mentha longifolia, A.m=Achillea millefolium

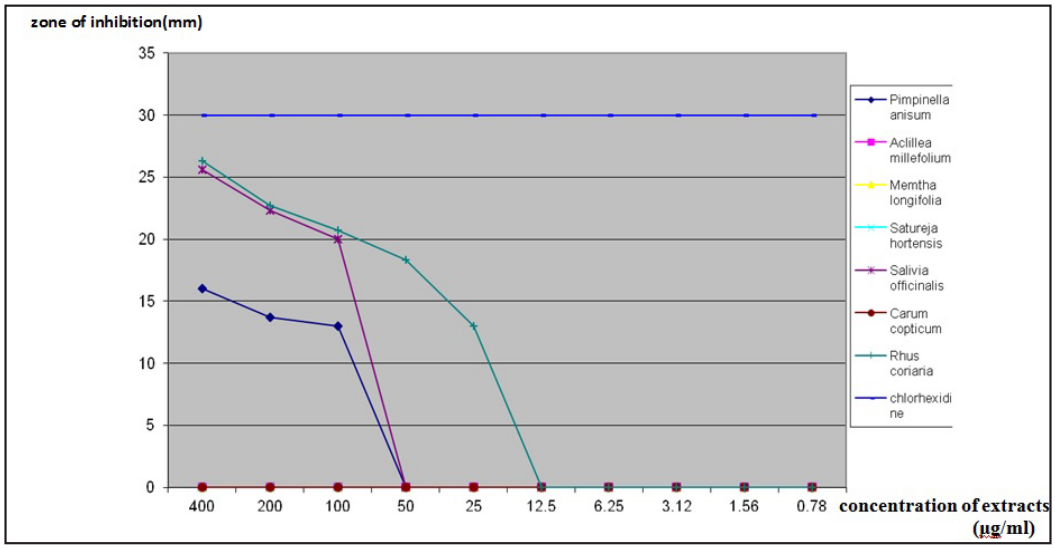

Figure 1. *The Size of zone of inhibition (mm) of extracts for Streptococcus mutans.*

For *L. rhamnosus,* only *P. anisum, C. copticum* and *R. coriaria* made zone of inhibition that their diameter in 100, 200 and 400 µg/ml for *R. coriaria* were significantly more than *P. anisum* (P<0.001) and *P. anisum* was more than *C. copticum* (P=0.038). *C. copticum* made zone of inhibition only in 400 µg/ml. In 50 and 25 µg/ml, diameter of zone of inhibition for *P. anisum* was significantly more than *R. coriaria* (P=0.002) and *C. copticum* (P=0.001). Lower concentrations didn't make any zone of inhibition (*Figure 2*).

For *A. viscosus* only the extracts of *M. longifolia*, *R. coriaria* and *C. copticum* made some zone of inhibition which their diameter in 200 and 400 µg/ml for *M. longifolia* was significantly more than *R. coriaria* (P<0.001) and *R. coriaria* was more than *C. copticum* (P<0.001) (*Figure 3)*. In 50 µg/ml, zone of inhibition for *M. longifolia* was significantly more than *R. coriaria* (P=0.037) and *R. coriaria* was more than *C. copticum* (P=0.006). In 50 µg/ml only *M. longifolia* made zone of inhibition which was significantly more than *C. copticum* (P=0.004) and *R. coriaria* (P<0.001). Lower concentrations didn't make any zone of inhibition.

Discussion

In this study, two experimental methods have been used to evaluate the antibacterial effect of seven of Iran's native plants against three cariogenic microorganisms. All extracts from evaluated plants have shown antibacterial effect against these three cariogenic bacteria by broth macrodilution method, but not by agar diffusion method in all extracts. That might be because the confounding factors that influence the result of agar diffusion methods are more than broth dilution methods; e.g. diffusion ability of antimicrobial agent through the agar

which depends on its nature [32,33]. By broth macrodilution method the inhibitory effect of *S. officinalis* was the highest which was followed by *M. longifolia, S. hortensis, C. copticum, R. coriaria, A. millefolium* and *P. anisum*. Therefore, our first hypothesis was proved to be right nevertheless, the second one was rejected.

In this method *S. officinalis* which was also herbal positive control had the best antibacterial effect against the three evaluated bacteria. *S. officinalis* is a well-known plant which its antimicrobial effects have been approved by many researches [23-29]. The phytomedicine texts have mentioned the usage of *S. officinalis* to treat anorexia, mouth and throat infections and increased perspiration [14]. There are some evidences of its antibacterial, antifungal and antiviral effect [14,15,24-29]. There are alpha and beta thujone (26-60%), 1,8 cineol (6-16%) and other flavonoids like epigenin in *S. officinalis* [14]. There are also some agents like linalool, borneol and α, β-caryophyllene in essential oil of *S. officinalis* [14]. In previous studies the effect of hydro-alcoholic extract of the leaf of *S. officinalis* against collagenolytic and the activity of *Porphyromunas gingivalis* was evaluated [15] and strong antibacterial and antifungal effects of *S. officinalis* against wide spectrum of bacteria like *Pseudomonas, Aspergillums* and *Candida* were reported [15,34] which was in agreement with the results of our research.

A. millefolium is one of the plants that has been used to treat the wounds, infected and gastroinstital disorders, and even to control the blood cholesterol for many years now [11,14]. There are some agents like chamazulene, caryophyllene, 1,8 cineol and some flavonoids like epigenin and rutin that are found in *A. millefolium* [14]. This plant had inhibitory and cidal effect against these evaluated microorganisms in our study which is similar to the results of Candon's research about antibacterial effect of essential oil of *A. millefolium* [16].

M. longifolia is a medicinal plant that is also used as food. It has been shown that the essential oil of different types of Mentha had strong antibacterial effects [35]. Metanolic extract of *M. longifolia* had also antibacterial and antifungal effects against wide spectrum of gram negative and gram positive bacteria and fungus [19]. In hydro-alcoholic extract of *M. longifolia* there are piperitone (60-80%), β – caryophyllene (5-15%), 1,8 Cineol (2-7%) and some flavonoids like hesperidin and quercitrin [14]. By broth macrodilution, the bactericidal effect of hydro-alcoholic extract of *M. longifolia* against *Lactobacillus* was significantly more than the well-known plant, *S. officinalis*; which shows the probable effect of the main effective agent – piperitone – against *Lactobacillus*. In addition, the effect of *M. longifolia* against *S. mutans* was

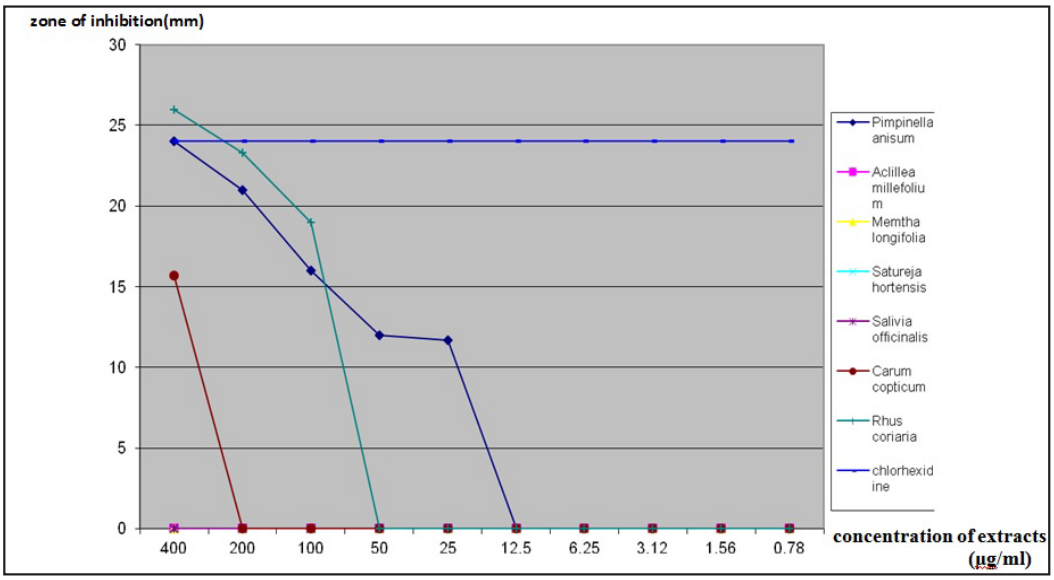

Figure 2. *The Size of zone of inhibition (mm) of extracts for Lactobacillus rhamnosus.*

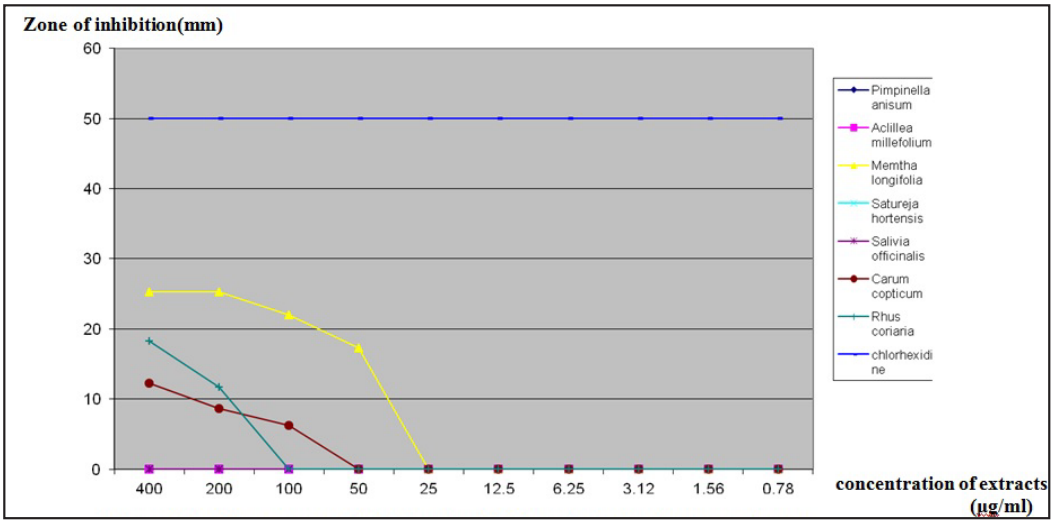

Figure 3. *The Size of zone of inhibition (mm) of extracts for Actinomyces viscosus.*

good and after *S. officinalis,* had the most inhibitory and cidal effect against that bacterium. On the other hand, it has been reported that *M. longifolia* didn't have any inhibitory effect against some *Streptococcus* like *S.* ***pyogenes*** **[35]**; therefore this effect seems important and needs more researches. The effects of *M. longifolia* against *A. viscosus* weren't the same as the two other cariogenic bacteria. It's probably because of the character of *A. viscosus* which is an anaerobic bacterium and produces some yellow colonies named "Sulfur Granule" in both tissue and aqueous environment. The higher resistance of this bacterium might be the result of being in these granules. In addition this bacterium needs blood and serum environment and doesn't grow very well in simple environments [36]. In addition, the effect of flavonoids in the plant should be concerned, especially the antibacterial effect of hesperidin which had been evaluated separately [37].

P. anisum is also a useful plant in traditional medicine of Iran. The common usage of this plant is for gastroinstital disorders, but it is used for superior respiratory organ disorders, fever and mouth and throat infections, as well [14]. Acetonic extract of *P. anisum* is effective for growth inhibition of some bacteria like ***Escherichia coli*** and ***Staphylococcus aureus*** and its essential oil has good effect against different spectrum of bacteria like *Salmonella typhi* and *E. coli* **[34].** The main agent of this plant is transe–anethole (94%) and there are β-caryophyllene and some flavonoids like epigenin and isovitexin in *P. anisum* [14,15]. In the present study *P. anisum* had good inhibitory effect against *S. mutans* and in higher concentrations against *Lactobacillus* and *A. viscosus* which are probably the result of the presence of anethol, β-caryophyllene and flavonoids.

In our research the effects of *P. anisum* is similar to bactericidal effect of aqueous extract of *P. anisum* against oral flora by disc diffusion method [38]. Probably, the hydrophilic agents in *P. anisum* play an important role in antibacterial effects.

S. hortensis is also a medicinal plant that is used as food and has antibacterial and antifungal effect against a wide spectrum of bacteria and fungus [34,39]. The essential oil of *S. hortensis* has mild antiseptic effect and also as it has Cymol and carvacrol in it, its aqueous extract has antiviral effect [14,34]. The main agents of *S. hortensis* are carvacrol and P- cyeme [14,34]. The phenolic type of carvacrol has a well-known antimicrobial effect [34,40,41], and doesn't dissolve in water. As the hydrophobic agents like carvacrol influence on cellular membrane of microorganisms and inhibit their growth [42]; and because of the hydrophobic nature of *S. hortensis* that prevents the diffusion in aqueous environment; therefore *S. hortensis* had moderate antibacterial effect in this study.

R. coriaria is a well-known plant in traditional medicine of Iran [10,13]. As long as we know, the antimicrobial effect of the fruit of *R. coriaria* hasn't been evaluated against cariogenic bacteria yet. The results of our study are similar to the results of another study about the effect of methanolic extract of the leaf of *R. coriaria* against gram positive and gram negative bacteria and *Candida* [43]. But, it should be considered that the agents in the leaf and fruit of *R. coriaria* like β-caryophyllene, caryophyllene oxide and triponoid agents are different. *R. coriaria* had good inhibitory and cidal effect against all three cariogenic bacteria with both experimental tests. Probably, the antibacterial effect may be from tanens like tannic acid (13-27%) and gallic acid. The presence of some agents like β-caryophyllene and α-terpineol in *R. coriaria* and *M. longifolia* is an important issue; especially according to the results of its bactericidal effect against *Lactobacillus*, which probably shows the special effect of these agents against *Lactobacillus*. This effect is very important, because the MBC of *M. longifolia* and *R. coriaria* was the same. On the other hand, presence of acidic agents may limit the usage of this plant to prevent dental biofilm plaque formation; but we can use it after excluding the acidic agents by some solvents like ether – 2 – petrol. The main agents of *R. coriaria* are β-caryophyllene, **terpenoid** agents, **monosesqui** and diterpenes [16].

C. copticum is a plant which is used for **gastrointestinal** disorders in Iran`s traditional medicine [10,13]. It is the only member of Apiaceae family which has thymol and carvacrol and from this perspective it is similar to *S. hortensis*. The effect of hydro-alcoholic extract of *C. copticum* against three cariogenic bacteria is probably reported for the first time in this study; but the effect of essential oil of *C. copticum* against different spectrum of gram positive and gram negative bacteria and dermatitis was evaluated in the past [34]. According to the presence of the same agents like carvacrol and γ-cymene in essential oil and hydro-alcoholic extract, probably these two agents can be the antibacterial agents.

The other important point is the presence of similar agents -caryophyllene- in *P. anisum*, *M. longifolia*, *R. coriaria* and *A. millefolium* [14,15]. In addition there are 1,8 cineal in *M. longifolia*, *A. millefolium* and *S. officinalis* [14,15] and they can probably be the effective antibacterial agents.

The differences between two methods -broth macro dilution and agar well diffusion- is probably because of the presence of polar agents which are better released in the aqueous environment of broth dilution method and show the antibacterial effect. This difference can help us to identify the group of effective agents according to their polarity.

Based on the fact that all of the extracts showed antibacterial effect by broth macrodilution method; the plant that didn't make zone of inhibition by agar diffusion method, may probably show antibacterial effect in higher concentration because of more proper concentration gradients in solid environment.

Some of the plants used in this study such as *S. hortensis, R. coriaria, M. longifolia* can also be used as food; therefore their consumption in high risk patients like the patients who have xerostomia or those who suffer from severe decay due to excessive numbers of radiotherapy, can be useful and without any harm. Meanwhile, the world's attraction to traditional treatment and the necessity of having medicines derived from plants, and microbial resistance to chemical drugs; preparing some products like antimicrobial mouthwashes from these plants, after animal and human studies to determine the therapeutic dose, seem to be beneficial and necessary.

Conclusion

Despite all limitations of our study, the final results show

that all of the plants had growth inhibitory effect against cariogenic bacteria in broth macrodilution method and *S. officinalis* had the greatest growth inhibitory effect against all three cariogenic bacteria. All of the extracts, except *C. copticum*, also had bactericidal effect in the evaluated range of concentration. By agar diffusion method *R. coriaria* had antibacterial effect against all three cariogenic bacteria.

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References

1. Roberson T, Heymann H, Swift E. Sturdevant's art and science of operative dentistry. USA: Mosby Elsevier; 2013.

2. Walsh L. The current status of low level laser therapy in dentistry, Part 1. Soft tissue applications. *Australian Dental Journal.* 1997; **42**: 247-254.

3. Fabricant D, Farnsworth N. The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*. 2001; **109**: 69.

4. Farnsworth NR. (Editor) The role of ethnopharmacology in drug development. Wiley Online Library; 1990.

5. Jahān MAKN. Iksīr-i A'zam; 1917.

6. Prabu G, Gnanamani A, Sadulla S. Guaijaverin–a plant flavonoid as potential antiplaque agent against Streptococcus mutans. *Journal of Applied Microbiology*. 2006; **101**: 487-495.

7. Fejerskov O, Kidd E. Dental Caries: The Disease and Its Clinical Management. Copenhagen, Denmark: Blackwell Munksgaard; 2008.

8. Summitt JB, Robbins JW, Schwartz RS, dos Santos J. Fundamentals of operative dentistry: a contemporary approach. Quintessence Publishing; 2006.

9. Jahān MAKN. Ghrabadin-i A`zam. (1st edn.). Delhi: Nami Monshi Nolkshur; 1936.

10. Aghili Khorasani MH. Makhzan al Advieh. Tehran: Bavardaran Press; 2001.

11. Zakaria Razi AM. Al Havi fe-teb. Bombay: Osmani press, 1886.

12. Akhaveini Bokhari A. Hedayat al Moteallemin fe-teb. Mashhad: Ferdosi University press; 1992.

13. Ibn Sīnā H. Ghanun fe-teb. Tehran: Sorush press; 1998.

14. LaGow B. *PRD* for herbal medicines. Australia: Thomson PRD publications; 2005.

15. ESCOP. Phytotherapy ESCo. ESCOP monographs: the scientific foundation for herbal medicinal products. Germany: Thieme; 2003.

16. Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, et al. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp. millefolium Afan. (Asteraceae). *Journal of Ethnopharmacology*. 2003; **87**: 215-220.

17. Singh G, Kapoor I, Pandey S, Singh U, Singh R. Studies on essential oils: part 10; antibacterial activity of volatile oils of some spices. *Phytotherapy Research*. 2002; **16**: 680-682.

18. Shojaii A, Abdollahi Fard M. Review of Pharmacological Properties and Chemical Constituents of Pimpinella anisum. *ISRN Pharmaceutics*. 2012; 2012: 510795.

19. Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D et al.(2007) Antimicrobial and antioxidant properties of the essential oils and methanol extract from Mentha longifolia L. ssp. longifolia. *Food Chemistry.* 2007; **103**: 1449-1456.

20. Güllüce M, Sökmen M, Daferera D, Agar G, Özkan H et al. In vitro antibacterial, antifungal, and antioxidant activities of the essential oil and methanol extracts of herbal parts and callus cultures of Satureja hortensis L. *Journal of Agricultural and Food Chemistry*. 2003; **51**: 3958-3965.

21. Sabzghabaee AM, Davoodi N, Ebadian B, Aslani A, Ghannadi A. Clinical evaluation of the essential oil of "Satureja Hortensis" for the treatment of denture stomatitis. *Dental Research Journal (Isfahan)*. 2012; **9**: 198-202.

22. Rani P, Khullar N. Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant Salmonella typhi. *Phytotherapy Research*. 2004; **18**: 670-673.

23. Weckesser S, Engel K, Simon-Haarhaus B, Wittmer A, Pelz K, et al. Screening of plant extracts for antimicrobial activity against bacteria and yeasts with dermatological relevance. *Phytomedicine*. 2007; **14**: 508-516.

24. Bozin B, Mimica-Dukic N, Samojlik I, Jovin E. Antimicrobial and antioxidant properties of rosemary and sage (Rosmarinus officinalis L. and Salvia officinalis L., Lamiaceae) essential oils. *Journal of Agricultural and Food Chemistry*. 2007; 55: 7879-7885.

25. Hayouni EA, Chraief I, Abedrabba M, Bouix M, Leveau JY, et al. Tunisian Salvia officinalis L. and Schinus molle L. essential oils: Their chemical compositions and their preservative effects against Salmonella inoculated in minced beef meat. International journal of food microbiology 2008; **125**: 242-251.

26. Sanei AS, Pour Esmaeeli HR, Ebadifar A, Madahi A, Saboor B, et al. Antimicrobial effect of seven plants extraction on a few of pathogen microorganism of oral cavity. *Pajuhande Journal*. 1997; **2**: 9-15.

27. Miguel G, Cruz C, Faleiro M, Simões M, Figueiredo A, et al. Salvia officinalis L. essential oils: effect of hydrodistillation time on the chemical composition, antioxidant and antimicrobial activities. *Natural Product Research*. 2011; **25**: 526-541.

28. Royo M, Fernández-Pan I, Maté JI. Antimicrobial effectiveness of oregano and sage essential oils incorporated into whey protein films or cellulose-based filter paper. *Journal of the Science of Food and Agriculture*. 2010; **90**: 1513-1519.

29. Rodrigues MR, Kanazawa LK, das Neves TL, da Silva CF, Horst H, et al. Antinociceptive and anti-inflammatory potential of extract and isolated compounds from the leaves of Salvia officinalis in mice. *Journal of Ethnopharmacology.* 2012; **139**: 519-526.

30. MacFaddin JF, Mac Faddin JF. Biochemical Tests for Identification of Medical Bacteria. Philadelphia: Lippincott Williams & Wilkins; 2000.

31. Bakri I, Douglas C. Inhibitory effect of garlic extract on oral bacteria. *Archives of Oral Biology*. 2005; **50**: 645-651.

32. Ncube N, Afolayan A, Okoh A. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology*. 2012; **7**: 1797-1806.

33. Winn WC, Koneman EW. Koneman's color atlas and textbook of diagnostic microbiology: Philadelphia: Lippincott Williams & Wilkins; 2006.

34. Ray A, Sarma B, Singh U. Medicinal properties of plants: Antifungal, antibacterial and antiviral activities. Chicago: International Book Distributing Co; 2004.

35. Mimica-Dukić N, Bozin B, Soković M, Mihajlović B, Matavulj M. Antimicrobial and antioxidant activities of three Mentha species essential oils. *Planta Medica*. 2003; **69**: 413-419.

36. Collier LB, A Susman M. Topley & Wilson's Microbiology and Microbial infections, Vol 5- Parasitology; 1998.

37. Lio M, Uyeda M, Nakagawa Y. Flovonoids as a possible preventive of dental caries. *Agricaltural and Biological Chemistry*. 1984; **28**: 2143-2145.

38. Chaudhry N, Tariq P. Bactericidal activity of black pepper, bay leaf, aniseed and coriander against oral isolates. *Pakistan Journal of Pharmaceutical Sciences*. 2006; **19**: 214-218.

39. Şahin F, Karaman I, Güllüce M, Öğütçü H, Şengül M, et

al. Evaluation of antimicrobial activities of Satureja hortensis L. *Journal of Ethnopharmacology*. 2003; **87**: 61-65.

40. Juven B, Kanner J, Schved F, Weisslowicz H. Factors that interact with the antibacterial action of thyme essential oil and its active constituents. *Journal of Applied Microbiology*. 1994; **76**: 626-631.

41. Kim J, Marshall M, Cornell J, WEI C. Antibacterial activity of carvacrol, citral, and geraniol against Salmonella typhimurium in culture medium and on fish cubes. *Journal of Food Science*. 1995; **60**: 1364-1368.

42. Ultee A, Kets E, Smid E. Mechanisms of action of carvacrol on the food-borne pathogen Bacillus cereus. Applied and Environmental Microbiology **65**: 1999; 4606-4610.

43. Iauk L, Caccamo F, Speciale A, Tempera G, Ragusa S, et al. Antimicrobial activity of Rhus coriaria L. leaf extract. *Phytotherapy Research*. 1998; **12**: S152-S153.