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Antimicrobial activity of aqueous and ethanolic extracts of *Heracleum persicum*, *Myrtus* and *Lemon verbena* against *Streptococcus mutans*

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ABSTRACT

Regarding prevalence of oral bacteria and their complications and effects on increase of bacterial resistance toward chemical medications, identification and application of herbals for fighting bacteria has been of crucial importance from the past. The aim of this study is to investigate the antimicrobial activity of aqueous and alcohol extract of *Heracleum persicum*, *Myrtus* and *Lemon verbena* against *Streptococcus mutans*. For this purpose, disk and well methods were applied and MIC and MBC of each extract were determined by broth micro-dilution method. In both methods, the best treatment of *Streptococcus mutans* was ethanol extract of *Myrtus* at 600 mg/mL concentration which did not have significant difference with the hallow diameter induced by Amoxicillin antibiotic (21/33). In this study the minimum inhibition concentration and minimum bactericidal concentration of *Myrtus* ethanolic extract were 3.12 mg/mL and 6.25 mg/mL, respectively. After that, *Lemon verbena* aqueous extract with 6.25 mg/mL and 12.5 mg/mL was in the second rank. Among the studied extracts, *Myrtus* ethanolic extract and aqueous extract of *Lemon verbena* were the strongest agents, respectively and it is recommended, after further investigation of medical formulation, to be used as a substitution for antibiotics as they have lower side effects. The can also be used as mouthwash and antibacterial chewing gums.

KEY WORDS: DISK DIFFUSION, MINIMUM BACTERICIDAL CONCENTRATION, MINIMUM INHIBITION CONCENTRATION, WELL

INTRODUCTION

History of oral diseases goes back to the human history. About 500 bacterial species exist in mouth some of which can cause oral infectious disease. Reduction in pathogenic oral microbes can be very important in improvement of oral ulcers and infections (Mozafari et al 1995). Dental cavity is an infectious disease; it means that bacteria play role in its creation. If hygiene measures were not properly taken, protein materials of the saliva would precipitate on the teeth and in case of contacting with food, the possibility of the bacterial growth

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and sedimentation will increase. By increase of bacterial population and more sedimentation of food particles and saliva content, dental plaque will be created. Bacteria of dental plaque, by degrading sugar materials, produce acids, the more the number of these bacteria, the higher the amount of acid will be. This acid may cause degradation of dent enamel and release its mineral content and therefore weakens.

In this content, *Streptococcus mutans* is the most common oral bacterium which is the main cause of dental cavity. High levels of *S. mutans* put the person in high risk group; these groups varies in each country and race according to amount of *S. mutans* which is a facultative anaerobic, gram-positive coccus commonly found in human oral cavity. In oral health domain, to reduce oral microbial flora and prevent from dental cavity and gingival disease, application of antimicrobial agents, especially herbal ones (due to lower side effects) is of crucial important are increasing as application of chemical drugs results in bacterial resistance and other side effects which may be even more dangerous than the disease itself, (Mimica 2010, Vogel 2011, Amin et al 2012 and Karikalan and Mohankumar (2016).

In recent years, application of herbal drugs has become more common in oral hygiene as they have antimicrobial, antifungal and anticancer effects and lower side effects (Ryan 2004). Extract of many herbs have effective compounds against fungi, bacteria, viruses, and insects and also show antioxidant behavior (Kordali 2005; Burt 2004). Therefore, considering therapeutic methods of Iranian traditional medicine, finding new pharmaceutical resources for oral disease treatment seems essential. Among these herbals, *Myrtus, Heracleum persicum* and *Lemon verbena* can be mentioned. *Myrtus* is an ever-green plant whose leaves have pharmaceutical effects. *Myrtus* has been locally used for treatment of herpes and an antiseptic agent and also to treat the nasal mucus inflammation. *Lemon Verbena* is a plant in pale green whose leaves have been used for therapeutic purposes its flower and leaves has been also used as tea and flavoring; its leaf essence is also employed for making perfumes. *Heracleum persicum* is an aromatic plant which has been long used for flavoring the foods. *Heracleum persicum* has numerous properties including strong antimicrobial and disinfecting agent. In this study, the effects of *Heracleum persicum, Lemon verbena* and *Myrtus* on *S. mutans* has been investigated and their ability in reduction or removal of harmful oral bacteria is examined.

MATERIAL AND METHODS

First, sampling was done; *Heracleum persicum*, *Myrtus* and *Lemon verbena* leaves were collected from Fars province and evaluated by an expert to identify them. Before, grinding, leaves were dried in shadow exposed to free air. Extraction was performed by wetting and then application of rotary machine. Ethanol 96% was used for preparing he ethanolic extract and distilled water was employed for preparation of aqueous extract. Standard strain of *S. mutans* (PTCC 1683) was provided from Persian Type Culture Collection in lyophilized form.

Then by application of a physiologic serum, a microbial suspension equals to 0.5 McFarland (1.5×10^{-8} *CFU/ml*) was prepared. Antibacterial test was performed by two methods of disk and well. Minimum inhibition concentration (MIC) was determined in 96-well microplates by standard broth micro-dilution method (dilution n liquid medium). Minimum bactericidal concentration (MBC) was determined based on MIC values of each extract. Statistical analyses of the data were performed by MINITAB 16 software. To examine significance of the effects and compare alcoholic and aqueous extracts in different concentrations, the mentioned software (one-way variance test based on Toochi test with $P<0.05$) was employed. All the measurements were repeated for three times and the results were reported in the form of average standard deviation.

RESULTS AND DISCUSSION

Results of well method are listed in table 1 as average diameter of *S. mutants* inhibition zone hallow ± standard deviation according to table 1 and P-values. All the data were normal. As table 2 shows, C7 related to antibiotic (amoxicillin) is in group A and C1, related to ethanolic extract of *Myrtus* against *S. mutants* is in group A and B; C4 including aqueous extract of *Heracleum persicum* is in group C. therefore, it can be said that amoxicillin and aqueous extract of *Myrtus* do not have significant difference. However, they both have significant difference with aqueous extract of *Heracleum persicum*. C6, C2, C5 and C3 included aqueous extract of *Lemon verbena*, aqueous extract of *Myrtus*, ethanolic extract of *Lemon verbena* and ethanolic extract of *Heracleum persicum* against S. mutans, respectively; and are located in group B and C. they don't have statistically significant difference with each other and also ethanolic extract of *Myrtus* and aqueous extract of *Heracleum persicum*; however, they have significant difference with amoxicillin antibiotic.

Results of well method are listed in table 3 as average diameter of s. mutants inhibition zone hallow standard deviation according to table 3, the data of zero imply that the extract had no impact on S. mutans. Based on the data of this table and P-values, all the data were normal. As it can be seen in table4, C7 including antibiotic (amoxicillin) against S. *mutans* is in group A and C1, related to ethanolic extract of *Myrtus* against *S. mutants*

Table 1. average diameter of *S. mutants* inhibition zone hallow (mm) standard deviation in three different concentrations of aqueous and ethanolic extracts of *Heracleum persicum*, *Myrtus* and *Lemon verbena* by well method.

extract	Concentration (mg/ml)					
	p-value	200	p-value	400	p-value	600
Ethanolic Myrtus	0.07	14±1.73	>0.15	14.6±71.52	>0.15	18.67±1.52
Aqueous Myrtus	>0.15	13±2	>0.15	13.33±1.52	0.07	14.33±0.57
ethanolic Heracleum persicum	>0.15	9	>0.15	11.33±1.52	>0.15	13.66±2.08
Aqueous Heracleum persicum	0.07	6.33±0.57	0.07	7.33±0.57	0.07	9.66±0.57
Ethanolic Lemon Verbena	0.07	10.67±1.15	>0.15	11.67±1.52	0.07	14.33±1.15
Aqueous Lemon Verbena	0.07	9.33±1.15	>0.15	14.67±2.08	0.07	17.33±0.57

Table 2. comparison of hallow diameter averages with each other and hallow induced by amoxicillin antibiotic by well method through Toochi method.

groups			average	number	
A			21.333	3	C7
A	B		15.780	3	C1
	B	C	13.777	3	C6
	B	C	13.553	3	C2
	B	C	12.223	3	C5
	B	C	11.330	3	C3
		C	7.773	3	C4

is in group A and B; C3 and C4 including ethanolic and aqueous extracts of *Heracleum persicum*, respectively, are in group C. Therefore, it can be said that amoxicillin and ethanolic extract of *Myrtus* do not have significant difference. However, they both have significant difference with aqueous and ethanolic extracts of *Heracleum persicum*. C5, C2 and C6 included ethanolic extract of *Lemon verbena*, aqueous extract of *Myrtus* and aqueous extract of *Lemon verbena* against S. mutans, respectively; and are located in group B and C. they don't have statistically significant difference with each other and also ethanolic extract of *Myrtus* and aqueous extract of *Heracleum persicum*; however, they have significant difference with amoxicillin antibiotic.

Regarding table 1 and 3, except aqueous extract of *Heracleum persicum* which had hallow diameter of 0 in three different concentration, the rest of extract showed antimicrobial behavior and the most effective treatment for gram positive bacterium of *S. mutans* in both methods (well and disk diffusion) was by ethanolic extract of *Myrtus* whose average hallow diameter in well method

Table 3. Average diameter of *S. mutants* inhibition zone hallow (mm) ± standard deviation in three different concentrations of aqueous and ethanolic extracts of *Heracleum persicum*, *Myrtus* and *Lemon verbena* by disk diffusion method.

Extract	Concentration (mg/ml)					
	p-value	200	p-value	400	p-value	600
Ethanolic Myrtus	>0.15	11.66±2.08	>0.15	13.33±2.51	>0.15	14±2.64
Aqueous Myrtus	>0.15	7±1	>0.15	8±1	>0.15	9±1
ethanolic Heracleum persicum	0.000	0.00	0.00	0.00	>0.15	8±2
Aqueous Heracleum persicum	0.00	0.00	0.00	0.00	0.00	0.00
Ethanolic Lemon Verbena	0.07	6.33±0.57	0.07	7.66±1.15	0.07	11±1.73
Aqueous Lemon Verbena	0.00	0.00	0.07	8.33±0.57	>0.15	11.33±1.52

Table 4. comparison of hallow diameter averages with each other and hallow induced by amoxicillin antibiotic by disk diffusion method through Toochi method.

groups			average	number	
A			21.333	3	C7
A	B		12.997	3	C1
	B	C	8.330	3	C5
	B	C	8.000	3	C2
	B	C	6.553	3	C6
	B	C	2.667	3	C3
		C	0.000	3	C4

and at concentration of 600 mg/ml was equal to 18.67 mm. this diameter was not significantly different with the hallow induced by amoxicillin (a common antibiotic) which was 21.33 ± 0.57 mm.

Numerous studies have reported antibacterial activity of herbal extracts. Many researchers have assigned antibacterial properties of *Myrtus* to its poly phenolic compounds (Cakir et al 2004).In a study by Montoro et al in 2005, the antimicrobial effects of *Myrtus* extract were attributed to poly phenolic compounds. This compound is often antibacterial and two important compounds named Mirtocomolone A and B would be released from that which have antimicrobial effects especially on gram positive bacteria such *as S. mutans* (Montoro et al 2005). Rotstein investigated the antibacterial effects of the compounds in Myrtus extract on gram positive and gram negative bacteria and shows that *Myrtus* has not a significant impact on gram negative bacteria and the antibacterial properties of this extract, especially on gram positive bacteria, could be attributed to Mirtokomolone A (Rotstein et al 1974). Gram negative bacteria, due to having saccharide lipo-poly external membrane and also some channels involving in material transportation, are inherently more resistant against toxins, hydrophilic dyes and antibiotics (Ebrahimi et al 2009). More sensitivity of gram positive bacterial to the extracts can be due to their different cell wall and its compound (Taskin et al 2007).

Saeidi et al (2012) investigated antibacterial properties of *Myrtus* extract and essence against antibiotic-resistant strains of *Staphylococcus aureus*. They concluded that high concentrations of *Myrtus* extract have antibacterial properties which are in complete agreement with the results of this study which examined the effect of high concentration of *Myrtus* extract on *S. mutans*. Therefore, these compounds can be applied for pharmaceutical therapies.The results of Carlo et al in 2010 about *Myrtus* indicated that the aqueous and ethanolic extract

Table 5. MIC and MBC values (in mg/ml) for ethanolic and aqueous extracts of *Myrtus*, *Heracleum persicum* and *Lemon verbena* against *Streptococcus mutans*.

Extract	MIC (mg/ml)	MBC (mg/ml)
Ethanolic *Myrtus*	3.12	6.25
Ethanolic Heracleum persicum	100	200
Ethanolic *Lemon verbena*	12.5	25
Aqueous *Myrtus*	12.5	25
Aqueous Heracleum persicum	100	200
Aqueous *Lemon verbena*	6.25	12.5

of this plant have highest percent of extractive compound. Also this extract has highest phenolic compound as well. As S. mutans showed highest sensitivity to the ethanolic extract, it can be concluded that its ethanolic extract has highest extractive compounds and phenolic compounds which can be an explanation for antimicrobial activity of *Myrtus* plant.

As table 1 shows, in well method and foe lower concentrations, the impact of aqueous and ethanolic extract of *Myrtus* on S. mutans did not differ, therefore, it can be said that in lower concentrations, *Myrtus* extract dissolved in water can have better impact. According to table 1 and 3, in both methods (disk and well) and among all the investigated treatments, gram positive bacterium of S. mutans exhibited the highest sensitivity to ethanolic extract of *Myrtus*; after that, aqueous extract of *Lemon verbena* had the highest impact; which did not have significant difference with the former extract this could be due to the type and compounds of aqueous extracts; which can explain the reason for different effective compounds of aqueous and ethanolic extract of *Lemon verbena*.

Due to possessing different metabolites in its essence (especially terpenoids and citral) *Lemon verbena*, has attracted considerable attention in terms of antimicrobial effects. Different studies have mentioned its effect in removal of oral microbial flora especially gram positive bacteria and controlling diarrhea. Terpenoids can be easily dissolved in different solvents such as water, methanol and ethanol (Zaferanieh 2003). Therefore, it can be claimed that in this research, water was more successful in dissolving terpenoids of *Lemon verbena* in high concentration therefore it was more effective on gram positive bacterium of S. mutans. Based on the data in table 1 and 3, in both methods of well and disk diffusion and in low concentrations of *Lemon verbena* extract, ethanolic extracts induced larger inhibition hallows in comparison with the aqueous extracts against *S. mutans* this can be explained by bactericidal activity of alcohol in lower concentrations.

In this research, regarding the values in tables 1 and 3, in both methods, *S. mutans* showed highest resistivity toward *Heracleum persicum* extract especially its aqueous extract which had significant difference with ethanolic extract of *Myrtus* and amoxicillin antibiotic. In disk diffusion test, in addition to aqueous extract of *Heracleum persicum*, its ethanolic extract had also significant difference with ethanolic extract of *Myrtus* and amoxicillin antibiotic.In disk diffusion method, aqueous extract of *Heracleum persicum* showed no inhibition zone against the growth of S. mutans for different concentration and only its ethanolic extracts showed antimicrobial properties in high concentrations. Nazemi et al in (2005) had examined antimicrobial activity of *Heracleum persicum* aqueous extracts against 5 pathogenic bacteria including *Bacillus subtilis, Bacillus polymixa, Staphylococcus, Nocardia aureus and Enterococcus faecalis* by disk diffusion test. *Heracleum persicum* extract and showed no inhibition hallow for the mentioned bacteria which is in complete agreement with the results of present study on *S. mutans*.

Regarding tables 1 and 3, the largest average hallow diameter that *Heracleum persicum* extract induced against *S. mutans* is related to its ethanolic extract obtained by well method which is equal to 13.66 in concentration of 600 mg/ml. In contrary to disk diffusion test, in well method, ethanolic extract of *Heracleum persicum* had no significant difference with ethanolic extract of *Myrtus* and amoxicillin antibiotic; however its antimicrobial activity was lower than the other extracts, but it still exhibited antimicrobial properties. Khorshidi et al (2014), proved existence of tannin and saponin compounds in Iranian *Heracleum persicum* by phytochemical tools. They also showed inhibitory effects of these compounds on *Escherichia coli, Salmonella antrydys*, *Staphylococcus aureus* and *Bacillus cereus*. They claimed that this herbal can be applied as an antimicrobial plant.

Useful pharmaceutical effects of plants are often due to their secondary products. In herbals, these compounds are mainly secondary metabolites such as alkaloids, steroids, tannins and phenolic compounds produced by the plant and restored in some of their specific parts. These compounds have antimicrobial properties in laboratory condition. Such complicated compounds can be found in specific groups, families and species (Balandrin et al 1985).

As figures 1 and 2 show, in both methods (well and disk diffusion), by increasing concentration of *Myrtus, Heracleum persicum* and *Lemon verbena*, antibacterial activity enhanced as increasing the concentration will increase the permeability of antibacterial extract agent to bacterium cell wall. Only aqueous extract of *Heracleum persicum*, with hallow diameter of zero, had no increase at any concentration which indicated the impact of solvent on extracting the effective compounds. Therefore, it can be said that water, in comparison with ethanol, has no ability to derive the effective compounds of *Heracleum persicum*. Generally, in this study, antibacterial activity decreased by reducing the concentration of applied extracts.

According to figures 3 and 4, *S. mutans* exhibited more sensitivity toward ethanolic extracts (except for *Lemon verbena* extracts whose aqueous extract had more antimicrobial properties at high concentrations) and showed larger hallows. Presence of alcohol resulted in derivation of more polar compounds; therefore, more sensitivity of gram positive bacteria can be attributed to polarity of extracts as polar compounds can easily penetrate through gram positive cell membranes and end to their death (i.e. *Streptococcus mutans*).

Streptococcus mutans showed sensitivity to all the extracts in well method which varied from 6.33 mm for

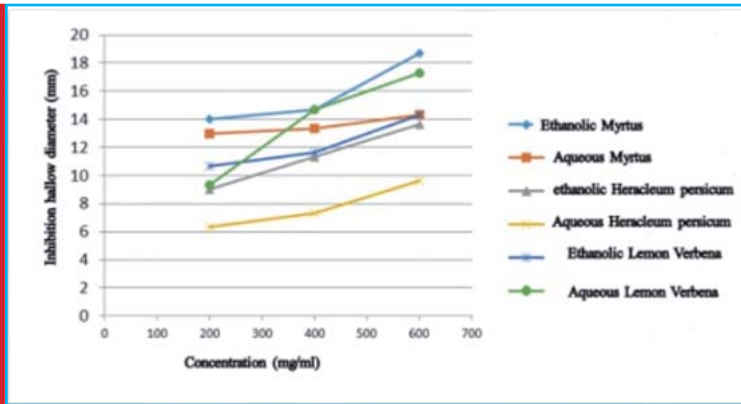

FIGURE 1. comparison of *Streptococcus mutans* growth inhibition hallow diameter for different concentrations of *Heracleum persicum*, *Myrtus* and *Lemon verbena* extracts by well method.

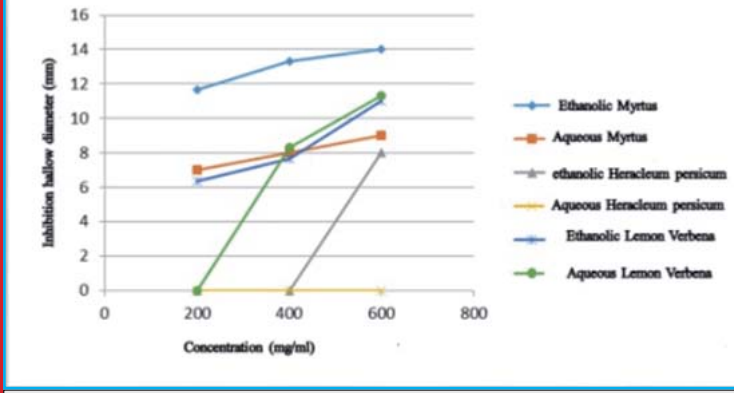

FIGURE 2. comparison of *Streptococcus mutans* growth inhibition hallow diameter for different concentrations of *Heracleum persicum*, *Myrtus* and *Lemon verbena* extracts by disk diffusion method.

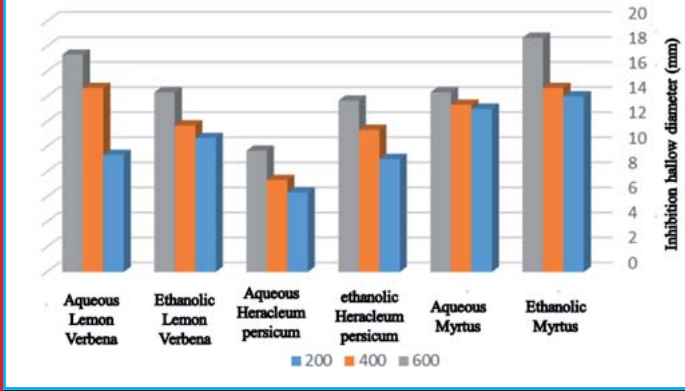

FIGURE 3. comparison of *Streptococcus mutans* growth inhibition hallow diameter for *Heracleum persicum*, *Myrtus* and *Lemon verbena* ethanolic and aqueous extracts by well method.

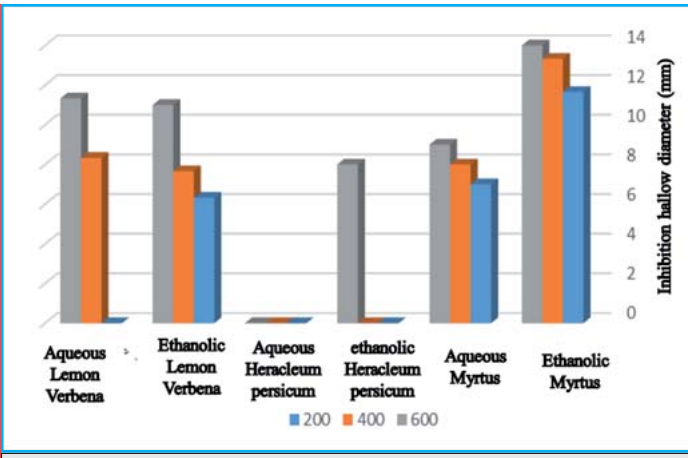

FIGURE 4. comparison of *Streptococcus mutans* growth inhibition hallow diameter for *Heracleum persicum*, *Myrtus* and *Lemon verbena* ethanolic and aqueous extracts by disk diffusion method.

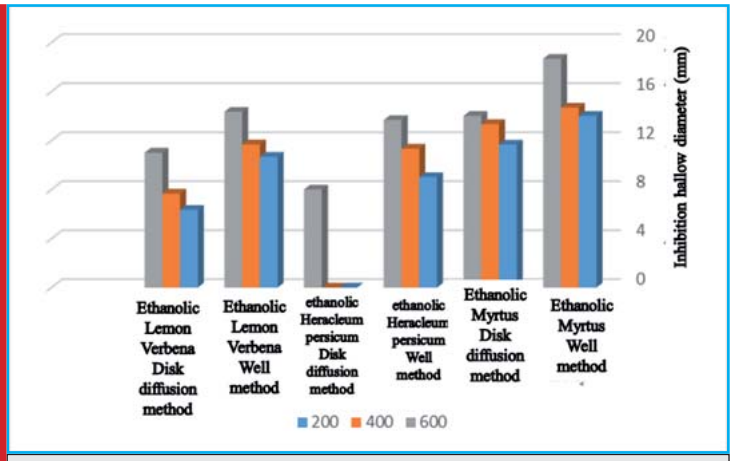

FIGURE 5. comparison of *Streptococcus mutans* growth inhibition hallow diameter obtained by both methods for different concentrations of *Heracleumpersicum*, *Myrtus* and *Lemon verbena* ethanolic extracts.

aqueous extract of *Heracleum persicum* to 18.67 mm for ethanolic extract of *Myrtus*. This trend didn't hold in disk diffusion test. According figures 5 and 6, extracts induced smaller inhibition hallows in comparison to well method; moreover, some extracts showed no antimicrobial activity against *Streptococcus mutans* in disk diffusion test and induced no hallow of growth inhibition. Quantitative comparison of these two methods indicated that well method, in comparison with disk diffusion scheme, showed higher inhibition effect on microorganism growth. This difference can be attributed to the fact that in well method, more amount of herbal extract is directly added to the wells while in disk diffusion test, the disks would be impregnated with the extracted for 15 minutes and put on agar surface indirectly. Therefore it is possible that the disks did not absorbed adequate amount of the extracts and do not show high propagation properties.

According to table 5, MIC of this study varied in 3.12-100 mg/ml range and variation range of MBC was 6.25-200 mg/ml. lower amounts would be neglected. It must be also noted that the reported MIC is related to MIC of the extracts. Hence, if the effective compounds of the extracts were separated, MIC values would definitely be lower than the reported values; as extract contains numerous compounds and only a few of them have antibacterial behavior.

For these experiments, double dilution series of the extracts were prepared in 96-well micro-plates in range of 0.09 mg/ml to 200 mg/ml. Among the studied treat-

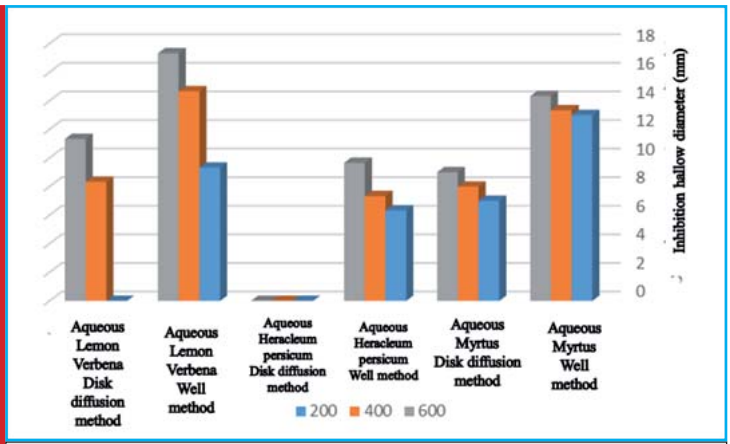

FIGURE 6. comparison of *Streptococcus mutans* growth inhibition hallow diameter obtained by both methods for different concentrations of *Heracleumpersicum*, *Myrtus* and *Lemon verbena* aqueous extracts.

ments, *Myrtus* ethanolic extract *Lemon verbena* aqueous extract exhibited lowest MIC (3.12 and 6.25 mg/ml) and lowest MBC (6.25 and 12.5 mg/ml) indicating higher sensitivity of *Streptococcus mutans* to this extract. Aqueous and ethanolic extracts of *Heracleum persicum* had highest MIC (100 mg/ml) and MBC (200 mg/ml), suggesting more resistance of *Streptococcus mutans* toward these extracts. These results coincide with the results obtained from disk diffusion and well methods. MIC and MBC of aqueous *Myrtus* extract and ethanolic *Lemon verbena* extract were the same and were equal to 12.5 mg/ml and 25/mg/ml, respectively.

CONCLUSION

Recently, side effects of antibiotics and microorganisms' resistance toward them have resulted in more attention to herbal extracts with specific biologic properties. Herbal-based antimicrobial compounds have numerous therapeutic uses. They are not only effective in treatment of infectious diseases, but they can simultaneously reduce some of the side effects of conventional antimicrobial agents. Therefore, regarding the significant impacts of herbals reported in traditional medicine, this study recommend that all these stages to be performed on other pathogenic oral bacteria and those exists in natural oral flora. In this way the optimized concentration of the mentioned extracts will be obtained which in addition to protecting form disease, do not disturb oral microbial balance. By collaborative cooperation of different centers, more comprehensive studies on this filed can be expected; which would have considerable impacts on treatment of antibiotic-resistant oral infections and reduction of oral health problems. Such studies can also pave way for mass production of herbal-based mouthwash and antibacterial chewing gums with minimum side effects.

REFERENCES

Amin, G. (2004) Most common Iranian traditional herbals, Tehran, Iran Medical Science University, First edition.

Balandrin, M.F.J., Kjocke, A., Wurtele, E. (1985) Natural plant chemicals: sources of industrial and medicinal materials. Science. 228: 1154-1160.

Burt S. (2004), Essential oils: their antibacterial properties and potential applications in foods—a review. International journal of food microbiology. 94(3):223-53.

Cakir, A., Kordali, S., Zengin, H. (2004), Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifolium* and *Hypericum heterophyllum*. Flavour Fragrance J, 19. Caries Res 40(5): 366-74.

Carlo Ignazio Giovanni Tuberoso, Antonella Rosa, Ersilia Bifulco, Maria Paola Melis, Angela Atzeri, Filippo Maria Pirisi, Maria Assunta Dessì, (2010), Chemical composition and antioxidant activities of *Myrtus communis L.* berries extracts, Food Chemistry, Volume 123, Issue 4, Pages 1242-1251.

Ebrahimi A., Khayami M, and Nejati V. (2009) Evaluation of antibacterial activity of ethanol extracts of fruit and oak disk diffusion method. Journal of Medicinal Plants. 33: 26-34.

Karikalan S. and A. Mohankumar (2016) Antibacterial efficacy of marine metalloenzyme against *Mutans streptococci* Biosci. Biotech. Res. Comm. 9(1): 109-113

Khorshidi M, Vaghef M and Bahmani B (2014), phytochemical effects and antimicrobial activity of Iranian *Heracleum persicum* extract, 22th National Congress on Food Industry and Science.

Kordali S, Kotan R, Mavi A, Cakir A, Ala A, Yildirim A. (2005) Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium, A. dracunculus, Artemisia santonicum,* and *Artemisia spicigera essential* oils. Journal of Agricultural and Food Chemistry.; 53(24):9452-8.

Mimica-DukicN, Bugarin D,GrbovicS, Mitic-CulaficD, Vukovic-GacicB, OrcicD. (2010). Essential oil of *Myrtus communis L.* as apotential antioxidant and antimutagenic agents. Molecules; 15(4): 2759-70.

Montoro P, Braca A, Pizza C, De Tommasi N. (2005) Structure antioxidant activity relationships of flavonoids isolated from different plants species. Food Chemistry; 92: 349-55.

Mozafari B, Mansouri Sh, Rajabalian S, Alimardani A and Mohammadi M (2005), In vitro study between anti-bacterial and cytotoxic effects of chlorhexidine and *Persica mouthrinses*. Journal of Dentistry department of Shahid Beheshti University of Medical Science, 3, 494-509.

Nazemi A. Hashemi M. Khatami Nezhad M. and Pourshamsian K (2005), first investigation of antimicrobial activity of *Heracleum persicum* aqueous and ethanolic extracts, medical science journal of Islamic Azad university, 2 (15), 91-94.

Rotstein A, Lifshitz A, Kashman Y. (1974) Isolation and antibacterial activity of acylphloroglucinols from *Myrtus communis linn*. Antimicrob Agents Chemother; 6(5); 539-42.

Ryan, KJ and Ray, CG. (editors) (2004), Sherris Medical Microbiology (4th ed.). McGraw Hill.pp.322-4, ISBN 0-8385-8529-9.

Saeidi s, Sabagh S K and Sabouri R E (2012), antimicrobial activity of *Myrtus communis linn* essence and extract against selective antibiotic-resistant strains of *Staphylococcus aureus*, Research Journal of Zabol Medical Science University.

Taskin E. Ozturk M and Kurt O. (2007) Antibacterial activities of some marine algae from the Aegean Sea (Turkey). Afr. J. Biotechnol. 6: 2746-2751.

Vogel CA, Boller K, Xue Y, Blair R, Aikens N, Burwick A. (2013) Learning As We Go: A First Snapshot of Early Head Start Programs, Staff, Families, and children.

Zaferanieh Z (2003) Effectiveness of Licorice, Chamomile and *Lemon verbena* extracts on *Tuberculosis mycobacterium*, MSc dissertation in microbiology Islamic Azad University, Lahijan, Iran.