Infection Epidemiology and Microbiology. 2018;4(2):67-72. DOI: 10.29252/modares.iem.4.2.67

Anti-Biofilm Activity of *Punica granatum*, *Ricinus communis*, and *Allium sativum* Plant Extracts on *Streptococcus mutans*

ARTICLE INFO

Article Type
Original Research

Authors
Shams S.* *PhD*,
Mehdipour A.[1] *PhD*,
Kermani S.[2] *MSc*,
Ghorbani H.[3] *BSc*,
Ragolia S.[4] *PhD*

How to cite this article
Shams S, Mehdipour A, Kermani S, Ghorbani H, Ragolia S. Anti-Biofilm Activity of *Punica granatum*, Ricinus communis, and *Allium sativum* Plant Extracts on *Streptococcus mutans*. Infection Epidemiology and Microbiology. 2018;4(2):67-72.

*Cellular & Molecular Research Center, Qom University of Medical Sciences, Qom, Iran
[1]Pediatric Dentistry Department, Dental Faculty, Qom University of Medical Sciences, Qom, Iran
[2]Cellular & Molecular Research Center, Qom University of Medical Sciences, Qom, Iran
[3]Student Research Committee, Qom University of Medical Sciences, Qom, Iran
[4]Molecular Medicine Department, University of Padova, Padova, Italy

Correspondence
Address: Cellular & Molecular Research Center, Qom University of Medical Sciences, Pardis Complex, Qom, Iran
Phone: +98 (25) 32854011
Fax: -
sshams@muq.ac.ir

Article History
Received: March 5, 2018
Accepted: April 25, 2018
ePublished: June 20, 2018

ABSTRACT

Aims *Streptococcus mutans* (*S. mutans*) is part of human oral cavity microbiome and is known to be responsible of dental caries. The aim of this study was to evaluate the inhibitory effects of *Punica granatum*, *Ricinus communis*, and *Allium sativum* extracts on biofilm formation caused by *S. mutans*.

Materials & Methods In this experimental study, the biofilm formation was carried out by broth dilution method with glucose -supplemented Tryptic Soy Agar (TSB) in 96-well microtiter plates. Seven serial dilutions from the aqueous extracts of the Punica granatum, Ricinus communis, and Allium sativum were prepared. Then, a suspension of S. mutans was added to the wells. The anti-biofilm effects of the extracts and turbidity were measured by an ELISA reader apparatus at OD492nm. Experiments were completed in triplicate.

Findings *Ricinus communis* was more active on *S. mutans* than other extracts. In comparison with others, the mean OD obtained in the presence of a concentration of 50mg of the plant extract (OD=0.083) was close to the negative control (OD=0.068). This plant was effective in higher concentrations (50, 25, 12.5 and 6.25mg/ml). *Allium sativum* extract has a moderate effect on *S. mutans*. The lowest activity belonged to *Punica granatum* extract.

Conclusion The extract of *Ricinus communis* has strong anti-biofilm activity against *Streptococcus mutans*, when compared to other extracts, *Allium sativum* extract show moderate activity on the biofilm formation. Aqueous extract of *Punica granatum* peel isn't very effective on *S. mutans*.

Keywords *Streptococcus mutans*; Biofilms; *Punica granatum*; *Ricinus communis*; *Allium sativum*

CITATION LINKS

[1] Role of VltAB, an ABC transporter complex ... [2] The virulence of Streptococcus mutans and ... [3] Damage of Streptococcus mutans biofilms by carolacton ... [4] Biofilms: A microbial ... [5] Penetration barrier contributes to ... [6] The role of chlorhexidine in caries ... [7] Traditional medicinal plant extracts and natural ... [8] Some medicinal plants with antiasthmatic potential ... [9] In vitro antibacterial activity of aqueous and ethanol extracts ... [10] Effects of Mikania genus plants on growth and ... [11] Antibacterial and antibiofilm activity of ... [12] In vitro assay for the anti-Brucella activity of medicinal plants ... [13] Therapeutic effects of Iranian herbal extracts against Trichomonas ... [14] Antimicrobial activity of pomegranate ... [15] Antioxidant and antibacterial potential of pomegranate ... [16] Physical, hematological, and histopathological signs ... [17] Antimicrobial potential of Ricinus communis ... [18] Characterization and evaluation of antibacterial ... [19] Helicobacter pylor--in vitro susceptibility to ... [20] Potent antifungal activity of garlic ... [21] Antibacterial effect of Allium sativum cloves ... [22] Therapeutic effects and applications of garlic and ... [23] Direct measurement of chlorine penetration ... [24] Penetration of antibiotics through Staphylococcus ... [25] Control of biofilm formation: Antibiotics and ... [26] Dental plaque as a biofilm and a microbial ... [27] Biofilm: A dental microbial ... [28] Degradation of 1, 2-dichloroethane by Ancylobacter aquaticus ... [29] Antibacterial effects of methanolic extracts of Zataria ... [30] Antibacterial effect of Methanolic extract of Camellia Sinensis L. on Pseudomonas ... [31] Chemical composition, mechanism of antibacterial action and antioxidant activity of leaf ... [32] Antibacterial activity of five Peruvian medicinal plants against Pseudomonas ... [33] Antibacterial activity against Streptococcus mutans ... [34] Antimicrobial and anti-biofilm activities of the methanol extracts of medicinal ... [35] In vitro antimicrobial activity of an experimenta ... [36] Antibacterial and antifungal activities and phytochemical ... [37] Antimycobacterial and antibacterial activity of Allium ... [38] Inhibitory activity of garlic (Allium sativum) extract ... [39] Antibacterial activity directed isolation of compounds from Punica ... [40] In vitro antibacterial activity of pomegranate juice and peel extracts on cariogenic ...

Introduction

Streptococcus mutans (*S. mutans*) is a gram-positive coccus, rod-shaped and facultative anaerobes, which commonly belongs to the normal microbial flora living in the oral cavity. *S. mutans*, as a biofilm-forming bacterium, is also considered to be the causative agent for initiation of dental decay in human [1].

Oral cavity conditions and the composition of the bacterial flora are effective in the pathogenicity of *S. mutans*. The biofilm formation or dental plaque on tooth surfaces is one of the virulence factors of the *S. mutans*. This structure represents complex bacterial communities and their productions and is well known as a physical barrier that inhibits the penetration of the antibacterial agents. Among Streptococcus spp., *S. mutans* has a higher ability to form biofilm than other bacteria in the oral cavity [2, 3].

Minor gingivitis and periodontitis can follow the formation of the biofilm, so effective plaque removal procedures are expected to prevent the development of them [4]. In addition, biofilm has been attributed as a barrier in the reduced penetration of the antibiotics and antibacterial solutions, e.g. chlorhexidine [5].

Although solutions containing cetylpyridinium chloride, chlorhexidine and amine fluorides are still used for caries prevention, due to toxicity, staining of teeth etc. They should not be recommended for long-term use [6, 7]. The application of different plant extract in the treatment of various diseases, especially infectious ones, is more and more frequently reported in different studies alongside traditional methods [8, 9].

The potentiality of medicinal plants against oral pathogens and anti-biofilm effects are also published [10, 11].

Various medical plants, because of environmental conditions and geographical locations, are cultivated in Iran. Even historical references relate to medical plants as a traditional treatment of the diseases [12, 13].

The pomegranate (*Punica granatum* L.) belongs to the family punicaceae. The plant is widely culturing as a native plant in some regions of South East Asia, the Mediterranean, the Americas and other parts of the world [14]. It is suggested that this plant can be a potential source of natural flavonoids, antioxidants, and phenols with remarkable antimicrobial effects [15].

Ricinus communis L. or castor oil plant is in family euphorbiaceae. The plant grows commonly in tropical and warm temperature regions. Apart from anticancer and anti-inflammatory activities, antimicrobial effects of *Ricinus communis* L. on some bacterial and fungal strains are confirmed [16-18].

Allium sativum (Garlic) belongs to the plant family liliaceous and genus allium and close to onion, shallot, and leek. Medicinal purposes of this plant have been documented e.g. antibacterial, hypoglycemic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, cardiovascular, and hormone-like effects [19-22].

The aim of this study was to evaluate the inhibitory effects of *Punica granatum*, *Ricinus communis*, and *Allium sativum* extracts on biofilm formation caused by *S. mutans*.

Materials and Methods

In this experimental study *S .mutans* ATCC 35668 was used as a standard strain that was incubated in 5% CO_2 at 37°C for 24h on Tryptic Soy Agar (TSA; ibresco; Iran). *Ricinus communis* (seed), *Allium sativum* and *Punica granatum* L. (peel) were collected from a grocery store in Qom, Iran. Plants were washed with distilled water and dried under the shade at room temperature for 10-12 day in the dark box. Next, all the plants were separately grounded by an electric grinder to produce a powder.

Then each plant was weighed and soaked in 100ml sterile distilled water on a rotator at dark for 24h. Whatman papers were used to remove large particles from the extracts. Finally, obtained extracts were kept in a refrigerator until required.

Biofilm inhibition activity: The biofilm formation was carried out by the microbroth dilution method in sterile 96-well flat-bottom polystyrene microtiter plates. Then, 100µL of Tryptic Soy Broth (TSB; Merck; Germany) supplemented by 1% glucose was poured into the wells of the microplate. 10µL volume of three concentrations (50, 25, 12.5, 6.25, 3.125, 1.562, and 0.781mg/ml) of the *Punica granatum*, *Ricinus communis*, and *Allium sativum* extracts were separately added to each wells. Finally, 100µL from standardized *S. mutans* (1.5×10^8cells/ml) were inoculated to all wells.

The TSB with bacteria (1:1) and TSB without the extracts and the bacteria were used as positive and negative controls, respectively. The plate was incubated anaerobically for 48h at 37°C.

After removing the contents of wells and washing with sterile physiological saline (PBS, pH=7.4), the attached bacteria to walls were fixed with 96% ethanol for 10min. The plate was dried and then wells were stained with 200µl of 1% crystal violet solution for 10min at room temperature. Additional washing with distilled water was slowly done to remove excess dye. The staining of attached bacteria was resolubilized with 96% ethanol and the OD of was evaluated by an ELISA reader Synergy HT (BioTek®Instruments Inc, Winooski, VT; USA) apparatus at 492nm. Experiments were completed in triplicate.

Findings

Ricinus communis was more active on the *S. mutans* than other extracts. In comparison with others, the mean OD obtained in the presence of a concentration of 50mg of the plant extract (OD=0.083) was close to the negative control (OD=0.068). This plant was effective in higher concentrations (50, 25, 12.5 and 6.25mg/ml).

Lower concentrations of *Ricinus communis* were similar to other extracts and without antibacterial activity.

The activity of the *Allium sativum* extract in the inhibition of the *S. mutans*-biofilm production was moderate and showed a slight reduction in ODs.

The concentrations 50, 25, and 12.5mg/ml (OD=0.112, 0.117, and 0.121, respectively) of the *Allium sativum* were effected on the bacteria.

Although obtained OD differences between the positive control and the highest concentration of *Punica granatum* were remarkable, it indicated the lowest effect on *S. mutans*. No difference of antibacterial activity of different concentrations of the *Punica granatum* extract was observed (Diagram 1).

Discussion

The aim of this study was to evaluate the inhibitory effects of *Punica granatum*, *Ricinus communis*, and *Allium sativum* extracts on biofilm formation caused by *S. mutans*.

Usually, the production of biofilm by microorganisms is a defense mechanism against antibiotics, antibacterial agents, etc. for example, antimicrobial compounds containing some reactive chlorine species such as hypochlorite, chloramines, etc. may be deactivated in the external layers of the biofilm [23].

With the biofilm formation, reduced activities from different antibiotics such as cefotaxime, β-lactams, tobramycin, tetracycline etc. on Staphylococcus spp. and *Pseudomonas aeruginosa* are also confirmed [24, 25].

It is estimated a 1000-1500 times greater resistance of microbial cells growing in a biofilm than the planktonic cells [26, 27].

Different studies have shown that tooth decay is associated with increases in the population of mutans streptococci and other acidogenic and aciduric bacteria. Among, *Streptococcus mutans* is known as a primary cause of dental caries in human. The caries can be accelerated by biofilm formation on the hard tissues of the oral cavity [28].

Today, pharmaceutical research looks at traditional medicine and in communities using plants in the treatment of various diseases and from them natural agents are more and more frequently tested and used [21].

The antimicrobial activities of a large number of medicinal plants have been evaluated and reviewed [29, 30]. The plants due to their natural properties and various chemical components may act as a new source of antibacterial drugs. They have a collection of mechanisms of action of the bacteria, viruses, fungi, parasites, etc. [31].

On the other hand, the researchers are more interested in the recognition of the potential of natural plants in medicine due to the emergence of the resistant pathogens to antibiotics which are as a problem in the health system [32].

In addition, some herbal medicines on *S. mutans* and anti-biofilm effects have been studied by a very large number of scientists in different area of the world, for example, *Salvadora persica*, *Camellia japonica*, *Thuja orientalis* etc. [33, 34].

In this study, we evaluated the effects of aqueous extracts of *Punica granatum*, *Ricinus communis*, and *Allium sativum* with 50, 25, 12.5, 6.25, 3.125, 1.562 and 0.781mg/ml concentrations of *S. mutans*. Among them, the results of the present study indicated that the extract of *Ricinus communis* has strong anti-biofilm activity. This plant was effective from high to medium concentrations (50, 25, 12.5 and 6.25mg/ml). Different studies were shown that *Ricinus communis* is an antibacterial extract.

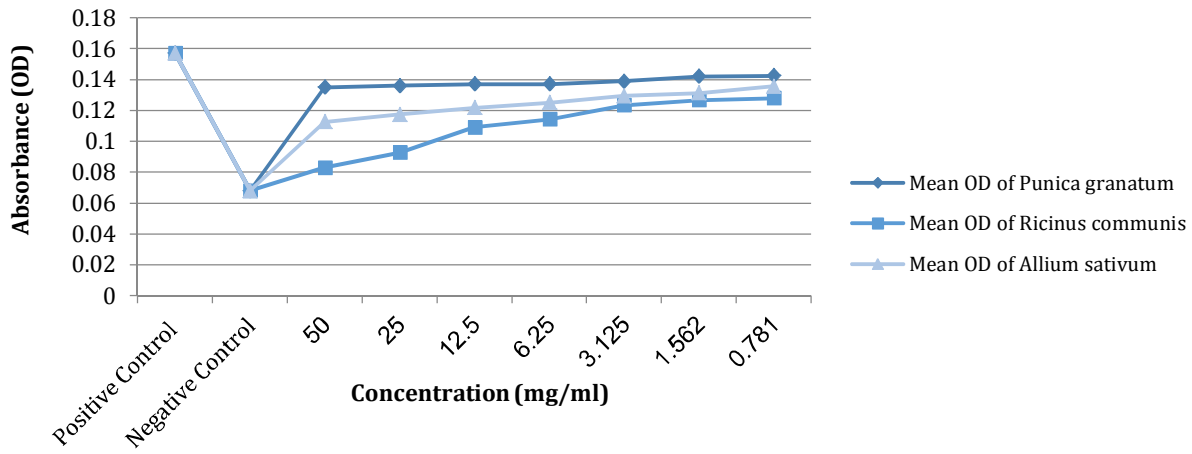

Diagram 1) Obtained curve from antibacterial activity of the plants against absorbance

Naz *et al*. A study using agar well diffusion and agar tube dilution methods of *Ricinus communis* on some bacteria and fungi such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Aspergillus fumigatus* and *Aspergillus flavus* showed that the methanol leaf extract was more active against bacteria than ethanol and aqueous extracts. Methanolic and aqueous extracts of the plant were also effective in fungal growth inhibition [17].

Another study by Leite *et al*. was done on antimicrobial activity of a *Ricinus communis* against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans*, *Enterococcus faecalis*, *Candida albicans* and *Candida glabrata*. They indicated that this plant at 2, 5 and 10% presented action against *S. mutans*, *S. aureaus* and *E. faecallis* [35].

Suurbaar *et al*. published that the used *Ricinus communis* extract had a major impact against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonaie*, and *Candida albicans*. The most activity was related to methanol extract of *Ricinus communis*, followed by ethanol and aqueous extracts [36].

Allium sativum extract showed moderate activity on the biofilm formation. It seems that the highest concentrations were effective (50, 25 and 12.5mg/ml). In evaluating the antibacterial activity of *Allium sativum* by Viswanathan *et al*. showed that the extract is an appreciable antimicrobial agent against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and some Mycobacterium spp. due to allicin and ajoene compounds [37].

Fani *et al*. also evaluated the inhibitory activity of *Allium sativum* on multidrug-resistant *S. mutans* isolated from human carious teeth. Their results showed that all isolates were sensitive to *Allium sativum* extract [38].

In the present study, in comparison with other extracts, aqueous extract of *Punica granatum* peel wasn't very effective on *S. mutans*. However, similar to some studies, a partial activity can be detected. Naz *et al*. identified methanolic extract of pomegranate fruit on against species of corynebacteria, staphylococci, streptococci, *Bacillus subtilis*, Shigella, Salmonella, *Vibrio cholera*, and *Escherichia coli*. In this study, Gram-positive bacteria were more sensitive to the plant [39].

Another study, evaluated peel extract of *Punica granatum* by Ferrazzano *et al*. could inhibit effectively the growth of *S. mutans* strain. It seems that this contrary is associated with used ethanolic extract by them [40].

In summary, the extract of *Ricinus communis* was shown to contain compounds with a strong anti-biofilm activity against, capable of inhibiting the growth of *Streptococcus mutans*. This plant extract could be a potential tool of new antimicrobial agents. More works into specific compounds within this extract and also *in vivo* studies are suggested in the future. Future studies are recommended to evaluate effective mechanisms of the extracts on biofilm.

The limitations of this research: Due to financial limitations, we were unable to extract the effective ingredient of the plants and to evaluate their effects on the target bacterium separately.

Conclusion

The extract of *Ricinus communis* has strong anti-biofilm activity against *Streptococcus mutans*, when compared to other extracts *Allium sativum* extract show moderate activity on the biofilm formation and aqueous extract of *Punica granatum* peel isn't very effective on *S. mutans*.

Acknowledgements: We thanks from Technology and Research Council of Qom University of Medical Sciences for supporting the study.

Ethical Permissions: No ethical permissions have been reported.

Conflicts of Interests: There is no conflict of interests.

Authors' Contribution: Shams S. (First author), Introduction author/ Methodologist/ Original researcher/ Statistical analyst/ Discussion author (35%); Mehdipour A. (Second author), Methodologist/ Original researcher/ Discussion author (25%); Kermani S. (Third author), Methodologist/ Assistant (20%); Ghorbani H. (Fourth author), Assistant (10%); Ragolia S. (Fifth author), Assistant/ Discussion author (10%)

Funding/Support: This work was supported by a grant from Technology and Research Council of Qom University of medical sciences.

References

1- Biswas S, Biswas I. Role of VltAB, an ABC transporter complex, in viologen tolerance in Streptococcus mutans. Antimicrob Agents Chemother. 2011;55(4):1460-9.

2- Krzyściak W, Jurczak A, Kościelniak D, Bystrowska B, Skalniak A. The virulence of Streptococcus mutans and the ability to form biofilms. Eur J Clin Microbiol Infect Dis. 2014;33(4):499-515.

3- Kunze B, Reck M, Dötsch A, Lemme A, Schummer D, Irschik H, et al. Damage of Streptococcus mutans biofilms by carolacton, a secondary metabolite from the myxobacterium Sorangium cellulosum. BMC Microbiol. 2010;10:199.

4- Chandki R, Banthia P, Banthia R. Biofilms: A microbial home. J Indian Soc Periodontol. 2011;15(2):111-4.

5- Singh R, Sahore S, Kaur P, Rani A, Ray P. Penetration barrier contributes to bacterial biofilm-associated resistance against only select antibiotics, and exhibits genus-, strain- and antibiotic-specific differences. Pathog Dis. 2016;74(6).pii:ftw056.

6- Autio-Gold J. The role of chlorhexidine in caries prevention. Oper Dent. 2008;33(6):710-6.

7- Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: Potential application in the prevention and treatment of oral diseases. Evid Based Complement Altern Med. 2011;2011:680354.
8- Taur DJ, Patil RY. Some medicinal plants with antiasthmatic potential: A current status. Asian Pac J Trop Biomed. 2011;1(5):413-8.
9- Venkatadri B, Arunagirinathan N, Rameshkumar MR, Ramesh L, Dhanasezhian A, Agastian P. In vitro antibacterial activity of aqueous and ethanol extracts of Aristolochia indica and Toddalia asiatica against multidrug-resistant bacteria. Indian J Pharm Sci. 2015;77(6):788-91.
10- Yatsuda R, Rosalen PL, Cury JA, Murata RM, Rehder VL, Melo LV, et al. Effects of Mikania genus plants on growth and cell adherence of mutans streptococci. J Ethnopharmacol. 2005;97(2):183-9.
11- Sánchez E, Rivas Morales C, Castillo S, Leos-Rivas C, García-Becerra L, Ortiz Martínez DM. Antibacterial and antibiofilm activity of methanolic plant extracts against nosocomial microorganisms. Evid Based Complement Altern Med. 2016;2016:1572697.
12- Motamedi H, Darabpour E, Gholipour M, Seyyed Nejad SM. In vitro assay for the anti-Brucella activity of medicinal plants against tetracycline-resistant Brucella melitensis. J Zhejiang Univ Sci B. 2010;11(7):506-11.
13- Fakhrieh Kashan Z, Delavari M, Arbabi M, Hooshyar H. Therapeutic effects of Iranian herbal extracts against Trichomonas vaginalis. Iran Biomed J. 2017;21(5):285-93.
14- Al-Zoreky NS. Antimicrobial activity of pomegranate (Punica granatum L.) fruit peels. Int J Food Microbiol. 2009;134(3):244-8.
15- Malviya S, Arvind, Jha A, Hettiarachchy N. Antioxidant and antibacterial potential of pomegranate peel extracts. J Food Sci Technol. 2014;51(12):4132-7.
16- Kuete V. Physical, hematological, and histopathological signs of toxicity induced by African medicinal plants. In: Kuete V. Toxicological survey of African medicinal plants. Amsterdam: Elsevier Science; 2014. pp. 635-57.
17- Naz R, Bano A. Antimicrobial potential of Ricinus communis leaf extracts in different solvents against pathogenic bacterial and fungal strains. Asian Pac J Trop Biomed. 2012;2(12):944-7.
18- Al-Mamun MA, Akter Z, Uddin MJ, Ferdaus KM, Hoque KM, Ferdousi Z, et al. Characterization and evaluation of antibacterial and antiproliferative activities of crude protein extracts isolated from the seed of Ricinus communis in Bangladesh. BMC Complement Altern Med. 2016;16:211.
19- Sivam GP, Lampe JW, Ulness B, Swanzy SR, Potter JD. Helicobacter pylor--in vitro susceptibility to garlic (Allium sativum) extract. Nutr Cancer. 1997;27(2):118-21.
20- Khan ZK, Katiyar R. Potent antifungal activity of garlic (Allium sativum) against experimental murine dissemenated cryptococcosis. Pharm Biol. 2000;38(2):87-100.
21- Karuppiah P, Rajaram S. Antibacterial effect of Allium sativum cloves and Zingiber officinale rhizomes against multiple-drug resistant clinical pathogens. Asian Pac J Trop Biomed. 2012;2(8):597-601.
22- Leuter H, Koch H, Lawson L. Therapeutic effects and applications of garlic and its preparation. In: Koch HP, Lawson LD. Garlic: The science and therapeutic application of Allium sativum L. and related species. Philadelphia: Williams & Wilkins; 1996. pp. 135-212.
23- De Beer D, Srinivasan R, Stewart PS. Direct measurement of chlorine penetration into biofilms during disinfection. Appl Environ Microbiol. 1994;60(12):4339-44.
24- Singh R, Ray P, Das A, Sharma M. Penetration of antibiotics through Staphylococcus aureus and Staphylococcus epidermidis biofilms. J Antimicrob Chemother. 2010;65(9):1955-8.
25- Algburi A, Comito N, Kashtanov D, Dicks LM, Chikindas ML. Control of biofilm formation: Antibiotics and beyond. Appl Environ Microbiol. 2017;83(3).pii:e02508-16.
26- Marsh PD. Dental plaque as a biofilm and a microbial community - implications for health and disease. BMC Oral Health. 2006;6(Suppl 1):S14.
27- Saini R, Saini S, Sharma S. Biofilm: A dental microbial infection. J Nat Sci Biol Med. 2011;2(1):71-5.
28- Ahn SJ, Ahn SJ, Wen ZT, Brady LJ, Burne RA. Characteristics of biofilm formation by Streptococcus mutans in the presence of saliva. Infect Immun. 2008;76(9):4259-68.
29- Hashemi A, Shams S, Barati M, Samedani A. Antibacterial effects of methanolic extracts of Zataria multiflora, Myrtus communis and Peganum harmala on Pseudomonas aeruginosa producing ESBL. J Arak Univ Med Sci. 2011;14(4):104-12.
30- Hashemi A, Shams S, Kalantar D, Taherpour A, Barati M. Antibacterial effect of Methanolic extract of Camellia Sinensis L. on Pseudomonas aeruginosa strains producing β-lactamases. J Gorgan Univ Med Sci. 2012;14(1):136-42.
31- Yang XN, Khan I, Kang SC. Chemical composition, mechanism of antibacterial action and antioxidant activity of leaf essential oil of Forsythia koreana deciduous shrub. Asian Pac J Trop Med. 2015;8(9):694-700.
32- Ulloa-Urizar G, Aguilar-Luis MA, De Lama-Odría MDC, Camarena-Lizarzaburu J, Del Valle Mendoza J. Antibacterial activity of five Peruvian medicinal plants against Pseudomonas aeruginosa. Asian Pac J Trop Biomed. 2015;5(11):928-31.
33- Wassel MO, Khattab MA. Antibacterial activity against Streptococcus mutans and inhibition of bacterial induced enamel demineralization of propolis, miswak, and chitosan nanoparticles based dental varnishes. J Adv Res. 2017;8(4):387-92.
34- Choi HA, Cheong DE, Lim HD, Kim WH, Ham MH, Oh MH, et al. Antimicrobial and anti-biofilm activities of the methanol extracts of medicinal plants against dental pathogens Streptococcus mutans and Candida albicans. J Microbiol Biotechnol. 2017;27(7):1242-8.
35- Leite VM, Pinheiro JB, Pisani MX, Watanabe E, De Souza RF, Paranhos Hde F, et al. In vitro antimicrobial activity of an experimental dentifrice based on Ricinus communis. Braz Dent J. 2014;25(3):191-6.
36- Suurbaar J, Mosobil R, Donkor AM. Antibacterial and antifungal activities and phytochemical profile of leaf extract from different extractants of Ricinus communis against selected pathogens. BMC Res Notes. 2017;10(1):660.
37- Viswanathan V, Phadatare AG, Mukne A. Antimycobacterial and antibacterial activity of Allium sativum bulbs. Indian J Pharm Sci. 2014;76(3):256-61.

38- Fani MM, Kohanteb J, Dayaghi M. Inhibitory activity of garlic (Allium sativum) extract on multidrug-resistant Streptococcus mutans. J Indian Soc Pedod Prev Dent. 2007;25(4):164-8.
39- Naz S, Siddiqi R, Ahmad S, Rasool SA, Sayeed SA. Antibacterial activity directed isolation of compounds from Punica granatum. J Food Sci. 2007;72(9):M341-5.
40- Ferrazzano GF, Scioscia E, Sateriale D, Pastore G, Colicchio R, Pagliuca C, et al. In vitro antibacterial activity of pomegranate juice and peel extracts on cariogenic bacteria. Biomed Res Int. 2017;2017:2152749.