Journal of Medicinal Plants Research Vol. 5(19), pp. 4870-4878, 23 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

In vitro antimicrobial effects of some herbal essences against oral pathogens

Vahabi S[1]*, Najafi E[2] and Alizadeh S[3]

[1]Periodontics Unit, Dental Faculty, Shahid Beheshti Medical University, Tehran, Iran.
[2]No: 23, Sonbol, North Pamchal street, Taleghani square, Azimieh, Karaj, Iran.
[3]Microbiology Department, Qazvin Medical University, Iran.

Accepted 13 May, 2011

Dental plaque, biofilms of microorganisms on tooth surface, plays an important role in the development of caries and periodontal disease. Our aim was to test *in vitro* antibacterial activity of some herbal extracts against *Actinomyces viscosus*, *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus fermentum*, *Lactobacillus casei* and *Eikenella corrodens*. Hydro alcoholic (50:50) extracts of 6 plants were taken using succilate method, then 4 dilutions of extracts (20, 40, 80, and 100% w/v, mg/ml) were put on blood agar media and cultured with one of the bacteria using 4 mm paper discs. Antimicrobial activities of these extracts were examined by disc diffusion method and mean of diameters of inhibition zone of each bacterium in different dilutions was reported. (P<0.05) hydro alcoholic extracts of *Punica granatum* and *Trigonella foenum-graceum* had strong antibacterial activity respectively, and their antibacterial activities were significantly less than Chlorhexidine and more than Irsha and Miswak. The hydro alcoholic extracts of *Scrophularia striata* and *Fumaria parviflora* showed less antibacterial activity in comparison with the first two and it was significantly less than Chlorhexidine and Miswak and more than Irsha. *Carthamus tinctorius* had the weakest antibacterial activity. We recommend more studies to demonstrate practical approaches of using natural materials on the oral biofilms.

Key words: Herbal extracts, antibacterial, dental plaque, chlorhexidine, essential oils.

INTRODUCTION

Periodontal disease is one of the most common health problems in the human communities (Loesche, 1996) and dental caries is still a common disease among children and adolescents (Alm, 2008). Many developed countries have shown a marked decrease in the prevalence of dental caries in children over the past decades, however, in many other developing countries caries prevalence has been increased (Al-Malik et al., 2006). Gingivitis is one of the most common forms of periodontal disease (Manson et al., 2000) and around 100% of people aged 17 to 22 have gingivitis in different degrees (Vahabi et al., 2007). Dental plaque, a biofilm of microorganisms on tooth surface, plays an important role in the development of caries and periodontal disease (Marsh, 1992). The accumulation and metabolism of bacteria on teeth and implants surfaces are considered the primary cause of caries, gingivitis, periodontitis, periimplantitis and breathe (Newman et al., 2006). The accumulation of plaque on teeth is a highly organized and ordered sequence of events (Theodore et al., 2006). Cariogenic bacteria and periodontopathic bacteria are present in dental plaque as biofilms (Takarada et al., 2004).

Essentially, all oral bacteria possess surface molecules that foster some type of cell-to-cell interaction (Newman et al., 2006). Only a few specialized organisms, primarily streptococci are able to adhere to oral surfaces such as the mucosa and tooth structure (Theodore et al., 2006). Mutans streptococci can colonize the tooth surface and initiate plaque formation by their ability to synthesize extracellular polysaccharides from sucrose, using glucosyltransferase (Jacquelin et al., 1995; Koo et al., 2000). This sucrose dependent adherence and accumulation of cariogenic streptococci is critical to the development of pathogenic plaque (Koo et al., 2000). All *Streptococcus mutans* serotypes such as *Streptococcus sobrinus* (serotypes d, g and h) have been shown to have significant potential to cause caries, but because of their

*Corresponding author. E-mail: isure1@gmail.com. Tel: 0098912 1992001. Fax: 009821 88560920.

significant genetic and biochemical differences, they should not be referred as simply as the single species *S. mutans* (Theodore et al., 2006). *S. mutans* and lactobacilli are acidogenic and acid uric bacteria and seem to be the primary organisms associated with caries in humans (Theodore et al., 2006). *S. mutans* are most strongly associated with the onset of caries, whereas lactobacilli are associated with active progression of cavitated lesions (Theodore et al., 2006). Bacterial attachment to preexisting plaque is studied by examining the adherence between different bacterial strains (co aggregation) (Newman et al., 2006). One of the best characterized interactions is the adherence of *Actinomyces viscosus* through surface fimberiae to polysaccharide receptor on cells of *Streptococcus sanguis* (Newman et al., 2006). These types of interactions are thought to be of primary importance in the colonization of the periodontal environment (Newman et al., 2006). The further accumulation of plaque around the gingival and subgingival region may lead to a shift in its microbial composition from streptococcus-dominated to a larger number of *Actinomyces* spp. and an increased number of capnophilic and obligatory anaerobic bacteria, such as *Porphyromonas gingivalis* (Marsh, 1994). Both streptococci and actinomycetes which are facultative anaerobes and seem to be involved in root caries and periodontal disease, respectively (Schüpbachet al., 1995; Slots et al., 1992), and doubling times for microbial populations during the first 4 h of development are less than 1 h (Newman et al., 2006). Consequently, these two groups of primary colonizers are taught to prepare a favorable environment for later (secondary) colonizers, which have more fastidious growth requirements (Newman et al., 2006). The microorganisms primarily considered secondary colonizers fell into the green (includes *Eikenella corrodens*), orange, or red complexes (Newman et al., 2006). *E. corrodens*, a fastidious, slow growing, gram negative and rod shaped bacteria that is part of the normal human oral flora, has been isolated from a variety of infections associated with human oral flora (Goldstein et al., 1983). This organism is implicated as a human periodontopathogen and may also cause extra oral infections (Chen et al., 1992).

Recent advances in microbiology and host defense studies allow clinicians to couple conventional mechanical therapy with locally and systemically delivered antimicrobial and host modulation agents (Newman et al., 2006). Mechanical procedures such as root planning is hard and takes usually more than one visit schedule and can cause wearing of enamel (Lindhe et al., 2008); moreover, improved understanding of the infectious nature of dental disease has dramatically increased interest in chemical methods of plaque control and holds great promise for advances in disease control and prevention (Newman et al., 2006). Chemical plaque control has been shown to be effective for both plaque reduction and improved wound healing after periodontal surgery; moreover it can augment mechanical plaque control procedures (Newman et al., 2006). Antimicrobial agents against oral microorganisms, especially those contributing to sub and supra gingival biofilm formation, play an important role in the prevention of dental caries, and periodontal disease (Groppo et al., 2008). Since some chemical materials including Chlorhexidine can cause brown staining of the teeth (Lindhe et al., 2008; Newman et al., 2006), tongue and silicate and resin restorations transient impairment of taste perception (Newman et al., 2006), toxic effects on connective tissues, dryness and soreness of oral cavity (Lindhe et al., 2008), allergic reactions in patients especially Asians (Ciancio, 1995) and oral desquamation in children (Almas, 2002), use of herbal agents can be a useful alteration.

Herbal products have been used since ancient times in folk medicine, involving both eastern and western medical traditions (Groppo et al., 2008). Many plants and plant-derived antimicrobial components are used in folk lone therapeutics for the treatment of periodontal disorders and for the purposes of oral hygiene (Tichy et al., 1998). Some have been evaluated for possible use in modern medicine, while thousands of other potentially useful plants have not been tested (Tichy et al., 1998). During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics has lead to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures which overcome the foregoing disadvantages (Enne et al., 2001; Marchese et al., 2001; Poole, 2001; WHO, 2001). A wide range of antimicrobial agents and herbal products are added to dentifrice and mouth rinsing solutions with the aim of preventing carries or biofilms formation (Groppo et al., 2008).

Several *in vitro* studies have indicated that *Salvadora persica* contains substances that possess dental plaque-inhibiting and antimicrobial properties against oral microbes (Manson et al., 2000; Almas, 1999; Baghie et al., 1994; Abdeirahman et al., 2002; Shahidi, 2004). Methanolic extracts of seeds of *Carthamus tinctorius* had antibacterial activity against *Bacillus cereus* (Shahidi, 2004), some gram-positive and some multi resistant bacteria (Mothana et al., 2008). Methanolic extract of *Punica granatum's* flowers had great antibacterial and antifungal effects (Haghighati et al., 2003), and the hydro alcoholic extract of its fruits was very effective against dental plaque microorganism (Menezes et al., 2006). Crude ethanolic extracts of leaves of *Trigonella foenum-graecum* demonstrated more antibacterial activity with less antifungal activity (Aqil et al., 2003).

Considering that only a few studies have been reported on the *in vitro* effect of these herbal extracts against oral pathogens and a great demand in dentistry for new and better substances to inhibit or suppress bacteria and biofilm formation, improve the quality of dental treatment,

Table 1. List of plants and their extracted parts used in the study with references to their source

Scientific name	English name	Vernacular name (in source)	Part extracted	Source
Salvadora persica	Tooth brush tree	Miswak	Roots	Saudi Arabia, Mecca
Punica granatum	Pomegranate	Golnar	Flowers	Iran, Karaj
Trigonella foenum-graecum	Fenugreek	Shanbalile	Seeds	Iran, Karaj
Scrophularia striata	Figworts	Teshnedary	Arial parts	Iran, Ilam
Fumaria parviflora	Fumitory	Shahtare	Arial parts	Iran, Karaj
Carthamus tinctorius	Safflower	Golrang	Flowers	Iran, Qazvin

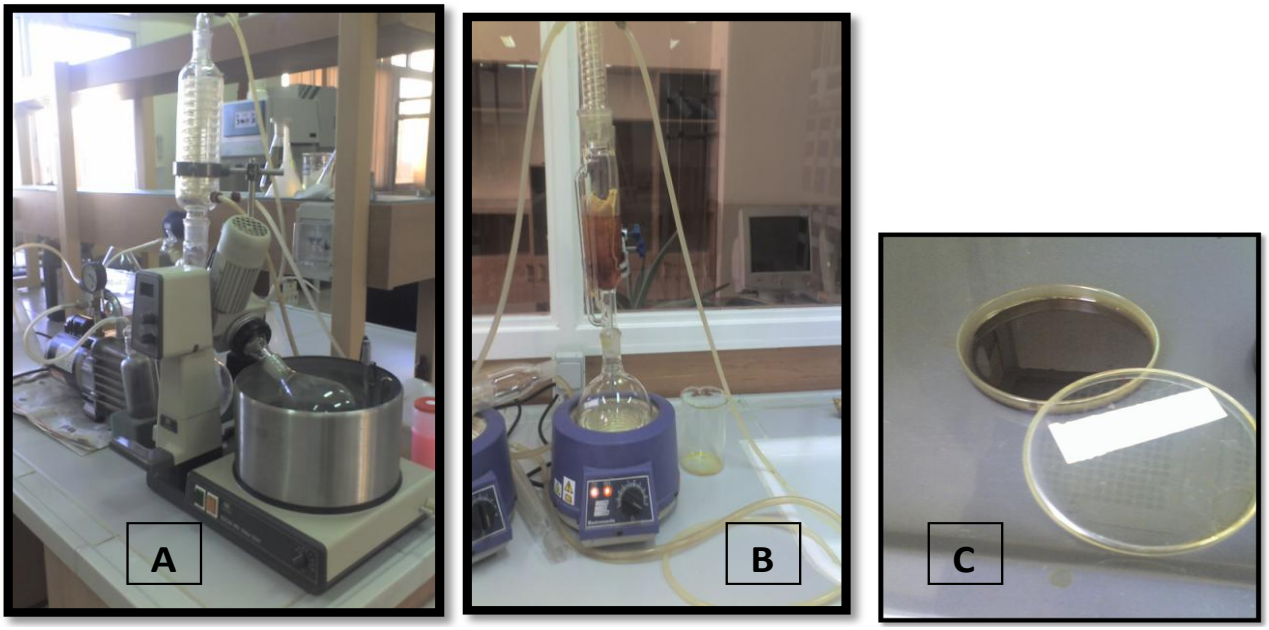

Figure 1. A: Soxhelet apparatus; B: Rotaevaporatory system; C: Plants extract after fully evaporation which is tar shape.

and facilitate some dental procedures; this study have been designed to evaluate the *in vitro* antimicrobial activity of these extracts on some oral micro organisms and compare them with Chlorhexidine, one essential oil mouth wash (Irsha) as the positive control and Miswak extract as the bench mark control.

METHODS

Preparation of the extracts

Plant species evaluated in the study are listed in Table 1. Six plant species, being used by Iranian native people, were collected from different regions of Iran and identified by pharmacognosy department, Tehran University, Iran.The air-dried plant parts powdered using a mechanical grinder and was extracted by ethanol- water solution (50:50) (Vahabi et al., 2007; Kartal et al., 2003), using a soxhlet apparatus (Figure 1A). In order to evaporate its water and alcohol, the rotaevaporatory system was used (Figure 1B). All extracts were kept in –20°C. Before starting the antimicrobial assay, extracts were soluted in distilled water in proportion of 1/20% weight to volume (w/v), this was the maximum concentration that could pass through the Millipore filter (30 mm in diameter, Orange scientific, Gyro disc CA-PC, FD0055-2), and then each solution was sterilized by the filters, gathered in sterile tubes and kept in –20°C.

Bacterial strains

The following 6 bacterial strains were used in this study: *A. viscosus* PTCC (Persian Type Culture Collection) 1202, *S. mutans* PTCC 1683, *S. sobrinus* PTCC 1601, *Lactobacillus fermentum* PTCC 1638, *Lactobacillus casei* subsp. casei PTCC 1608, and *E. corrodens* PTCC 1391; all bacterial strains were prepared in the form of standard and Lyophilized from IROST (Iranian Research Organization for Science and Technology).

Culture media and inoculums

We used Sheep blood agar base (Disco 241820) for *S. mutans, S. sobrinus and E. corrodens,* brain heart infusion agar (Disco 0045) for *A. viscosus* and Lactobacilli MRS broth for *L. fermentum* and *L. casei* (Disco 288130). Each bacterial strain was cultured in the

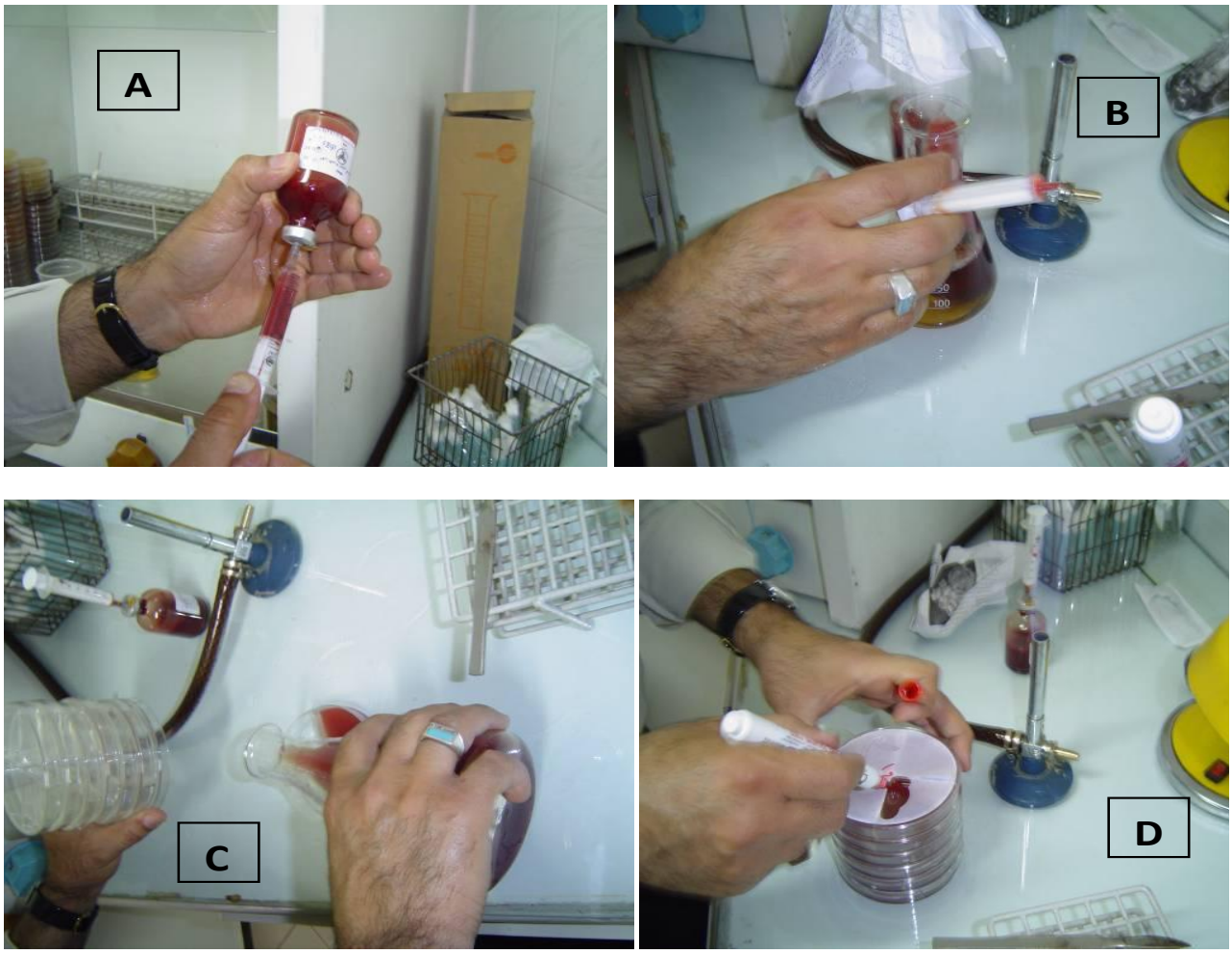

Figure 2. Different stages of preparing bacteria culture media: A: Sheep Blood; B: Preparing sheep blood agar media; C: pouring the prepared media in 10 mm diameter plates; D: Marking plates with different numbers.

suitable and specific condition determined by Iranian Research Organization for Science and Technology, after 18 to 24 h suspensions of bacteria which were adjusted spectro-photometrically to match the turbidity of a McFarland 0.5 scale, 1.5×10^8 Colony Forming Unit per milliliters (CFU/ml), were prepared for each strain, using sterile water. Suspensions was diluted 100-fold in sterile water and added to Blood agar media which was sterile and reached to 45°C. Inoculated media was poured in different plates (with the depth of 3 to 5 mm) and kept at temperature of 4°C for 24 h. (Figure 2A, B, C and D).

Assay of antibacterial activity

Screening plant extracts for their antibacterial activity was conducted using the agar disc diffusion method (Bauer et al., 1966; NCCLS, 2001; NCCLS, 2004). At first, bacteria were cultured in Sheep blood agar media, after 18 to 24 h, suspensions of bacteria (0.5 Mac Farland) were prepared using sterile water and then blood agar medias were inoculated by sterile swabs. After 15 m, 4 mm sterile blank paper disks were aseptically put on agar surfaces and immediately impregnated with different dilutions of extracts. The first blank disk was impregnated with 100% w/v of the extracts and the others with 80, 40 and 20% w/v, respectively. Plates were incubated for 18 to 24 h, then the zones of inhibition measured and the average of diameters noted. All these procedures were done for Chlorhexidine and Irsha mouth washes, also (Figure 3A, B).

Statistical analysis

The data from zones of inhibition of growth of each microorganism in different extracts concentrations were compared by non-parametric Kruskal-Wallis test. Statistical significance was determined at the level of $P<0.01$.

RESULTS

Salvadora persica

Among all six examined bacterial strains, it was more effective against *L. fermentum* and *A. viscosus* and its antibacterial activity against *S. sobrinus* was less than

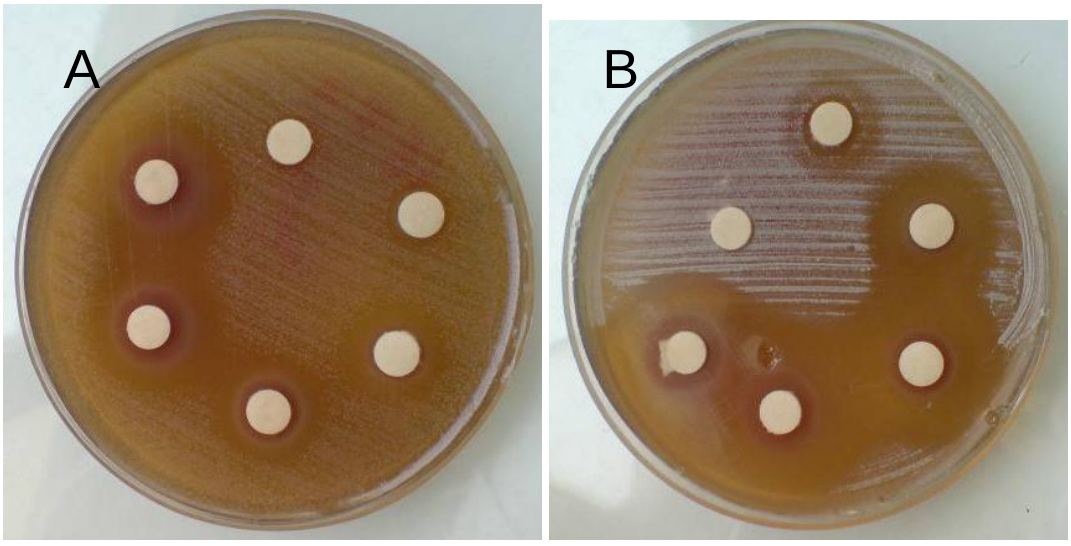

Figure 3. A: Zones of inhibition of Scrophularia extract against *S. mutans*, B: zones of inhibition of *P. granatum* extract against *E. corrodens*.

other bacteria. *S. persica* (Miswak) had the most antibacterial activity at 100% w/v. At this concentration its inhibition zones were significantly less than chlorhexidine and more than Irsha mouth wash in all bacteria examined.

Punica granatum

Among all six bacterial strains examined, it was more effective against *Eikenella corrodence* and *A. viscosus* and its antimicrobial effect against *L. fermentum* was less than other bacteria. Punica had the most antibacterial activity at 100% w/v, at this concentration its inhibitions zone were significantly less than chlorhexidine and more than Irsha and Miswak in all the bacteria examined, except for *E. corrodens* Punica's antimicrobial activity against *E. corrodens* was significantly more than chlorhexidine, Irsha and Miswak in all concentrations examined.

Trigonella foenum-graecum

It caused no zone of inhibition against *S. sobrinus* and it had just a slight antibacterial activity against *A. viscosus*. At 100% w/v (it's most effective concentration); its inhibition zone was significantly less than Chlorhexidine and more than Irsha (except for *S. sobrinus* which was less than Irsha). Trigonella's inhibition zone in *L. fermentum*, *L. casei* subsp. *casei*, *E. corrodencs* and *S. mutans* was significantly more than Miswak and in *A. viscosus* and *S. sobrinus* was significantly less than Miswak.

Scrophularia striata

At 100% w/v, its inhibition zones against all examined bacteria were significantly less than Chlorhexidine. Scrophularia 's zones of inhibition against *L. casei* and *L. fermentum* significantly were more than Irsha and Miswak, against *S. sobrinus* and *A. viscosus* was equal to Irsha and less than Miswak, against *S. mutans* was less than Miswak and more than Irsha and against *E. corrodens* was equal to Miswak and more than Irsha.

Fumaria parviflora

It caused no zone of inhibition against *E. corrodens*. At 100% w/v (it's most effective concentration); its inhibition zone was significantly less than Chlorhexidine. Fumaria's inhibition zones against *L. casei*, *L. fermentum* and *A. viscosus* were significantly more than Irsha and less than Miswak; against *S. mutans* and *S. sobrinus* were more than Miswak and Irsha and against *E. corrodens* were less than Irsha and Miswak.

Carthamus tinctorius

It caused no zones of inhibition against *A. viscosus*, *S. sobrinus*, *L. casei, L. fermentum.* At 100% w/v, its inhibition zones were less than Chlorhexidine against all bacteria; and significantly more than Miswak and Irsha against *S. mutans* and significantly more than Irsha and less than Miswak against *E. corrodens*.

DISCUSSION

The result of this study showed that totally the hydro

alcoholic extracts of *P. granatum,* and *T. foenum-graceum*, had strong antibacterial activity, respectively and their antibacterial activity was significantly less than Chlorhexidine and more than Irsha and Miswak. The hydro alcoholic extract of *Scrophularia striata* and *F. parviflora* showed less antibacterial activity in comparison with the first 2 ones and it was significantly less than chlorhexidine and Miswak and more than Irsha. *C. tinctorius* had the weakest antibacterial activity and it was significantly less than Miswak, Chlorhexidine and Irsha mouthwashes.

S. persica is a medical plant whose roots, twigs or stems have been used over centuries as oral hygiene tools in many parts of the world (Al-Sabawi et al., 2007) and has been reported to have many pharmaceutical effects such as anti plaque, anti caries, anti-inflammatory and antiviral properties (Almas, 1993; Daruot et al., 2000), even more, low dental caries among Miswak users has been reported in epidemiological studies (Almas, 2004). It was shown in different *in vitro* and *in vivo* studies that alcoholic and aqueous extracts of *S. persica* against different aerobic and anaerobic bacteria like *S. mutans* and *E. corrodens* had showed strong antimicrobial activity (Abdeirahman et al., 2002; Poureslami et al., 2007; Al-Bayati et al., 2008; Darout et al., 2008) which this result was in lined with our study, even the *in vivo* studies showed that using Miswak, Miswak extract and Persica mouth wash reduced salivary bacteria count and resulted in improved gingival health and lower carriage rate (Almas, 2004; Kalessi et al., 2004). Such antimicrobial effect of alcoholic extract of *S. persica* is believed to be due to contents of chlorides, tannins, trimetylaminesalvadorine, nitrate, thiocynate and sulphor (Almas, 1993; Darout et al., 2000). Sulfated compounds and isothiocyanate are known to be responsible for antibacterial effects of the plant, while fluoride and calcium salts are quite effective in preventing dental caries (Darout et al., 2000; Ezmirly et al., 1981; Darout et al., 2002). Trimethylamine is known in decreasing plaque accumulation. Tannins, tannic acid and benzyl isothiocyanate, are known to have antimicrobial effects and help the healing of gum inflammation (Ezmirly et al., 1981; Al Sadhan et al., 1999; Latif et al., 1995). Results of some studies reported low to moderate antimicrobial activity for *S. persica* ethanolic and water extracts (Almas, 1999; latif et al., 1995; Almas et al., 1999; Almas et al., 1999; Sofrata et al., 2008) and showed the Miswak pieces embedded in agar or suspended in the air above the agar plate clearly demonstrated much stronger inhibitory effects than the aqueous Miswak extract (Sofrata et al., 2008), in addition, Al-Sabawi et al. (2001) showed that inhibitory effect of ethanolic extract of *S. persica* is not significantly less than 0.2% Chlorhexidine (Al-Sabawi et al., 2007), which these two results are against our findings.

Punica granatum Linn. is a shrub or small tree native to Asia (Machado et al., 2002). Photochemical screening of ethanolic extract yielded positive results for sterols, flavonoids, triterpens, phenols and tannin is well established (Voravuthikunchai et al., 2005). The antibiotic activity of the extract of *P. granatum* is associated to tannin phytoconstituents and alkaloids found in leaves, roots, stem and fruits (Silva et al., 2008); there is a growing interest in using tannins as antimicrobial agents in caries prevention (Scalbert, 1991). The antimicrobial activity of *P. granatum* has been widely investigated (Menezes et al., 2006; Pereira et al., 2006; Vasconcelos et al., 2003; Muangsan et al., 2008). Ethanolic, water, Methanolic and acetone extract of *P. granatum* showed strong antimicrobial activity in different investigations done on both gram-positive and gram-negative non-oral bacteria (Haghighati et al., 2003; Machado Thelma et al., 2002; Voravuthikunchai et al., 2005, Silva et al., 2008; Muangsan et al., 2008; Reddy et al., 2007; Duraipandyan et al., 2006; Aqil et al., Rani et al., 2004; Naz et al., 2007; Negi et al., 2003; Meléndeza et al., 2006). Kakiuchi et al. (1986) and Pereira et al. (2006) examined a gel derived from *P. granatum,* the glucan synthesis and its antimicrobial action gave this gel an effective control of the already formed biofilm. Just a few researches done on oral bacteria; hydro alcoholic extract from *P. granatum* fruits showed very effective activity against dental plaque microorganisms in an *in vivo* study done by Menezes et al. (2006) had appropriate effects on the microorganisms in comparison with Chlorhexidine. All the studies were in accordance with ours, but studies which have been done on oral bacteria were rare.

T. foenum-graceum seeds (Billaud, 2001) and leaves (Sharma et al., 1996) are used as an ingredient in traditional medicine and have been reported to exhibit pharmacological properties which have different therapeutic effects, for example Saponins and Fenugreekine of its seeds introduced as anti-inflammatory, antiviral and antimicrobial elements of the plant (IHP, 2002). In our study, *T. foenum-graceum* extract was one of the strongest antibacterial extracts with inhibition zones between 0 to 18 mm. No report was found about the effect of *T. foenum-graceum* on oral bacteria. It was shown that *T. foenum-graceum* extract had a strong and broad spectrum antimicrobial activity (Shahidi, 2004), which was in lined with our study. Alzoreky et al. (2003) reported that *T. foenum-graceum* was not active (no inhibition zone) against any strains at tested concentrations (Alzoreki, 2003).

Different species of the genus *Scrophularia* (scrophulariaceae) have been used in traditional medicine to treat a wide diversity of disease, of which dermatosis (Viola, 1966) and inflammatory affections (Swiatek, 1970) stand out. Whereas no reports about *S. striata* were found, the extracts of other species of *Scrophularia* namely *Scrophularia nodusa* (Swiatek, 1970), *Scrophularia oldhami* (Won Sick Woo, 1963), *Scrophularia frutescens*, *Scrophularia sambucifolia* (Fernandez et al., 1996) and *Scrophularia ningpoensis*

(Tong et al., 2006) exhibited antibacterial and anti-inflammatory effect. In an investigation done by Stavri et al. (2006), antibacterial natural products of extracts of the whole plant of *Scrophularia deserti* were studied and 3 of the 8 components isolated from this plant exhibited antibacterial activity. In our study, *S. striata* showed moderate antibacterial activity against all the species examined, may be its antibacterial activity can be attributed to the presence of phenolic acids, like other species of this genus.

In the traditional medicine, lots of therapeutic benefits introduced for *F. parviflora* and its mouthwash was known to be useful in reducing gums inflammation (IHP, 2002). Antioxidant and antilipoperoxidant activities of Alkaloids and phenolic extracts of eight *Fumaria* species (include *F. parviflora*) was examined against some microorganisms (Souˇcek et al., 2007). For *F. parviflora*, no reports were found about its antimicrobial activity. However, Parekh and Chanda (2007) have evaluated antibacterial activity of Fumariaindica (Haussak) Pugsley's seeds aqueous and ethanol extracts against selected members of Enterobacteriaceae (Parekh and Chanda, 2007). In lined with our study, no strong antibacterial effect was found for Fumaria. None of the investigations screened the antibacterial activity of *C. tinctorius* on oral bacteria. It was reported that crude extract of *C. tinctorius* did not have very strong antimicrobial activity against some gram-positive bacteria and had no effect against gram-negative bacteria which this result is in lined with our study's (Mothana et al., 2008); however, our study this plant had moderate antibacterial activity against *E. corrodens* in comparison with our gram-positive bacteria. Mehrabian and Ramzi reported that the aqueous extract of *C. tinctorius* had the most microbiocidal effect in comparison with other solvents used (Mehrabian et al., 2000), which is against our results. This difference may be because of the differences in our solvents or bacteria we worked on.

Antimicrobial activity of Shiitak mushrooms (Hirasawa et al., 1999) and Bakuchiol (Katsura et al., 2001) extracts, herbal dentifrices (Lee Sean et al., 2004) and silver nitrate (Spacciapoli et al., 2001) were assayed against *Streptococcus* spp., *Actinomyce* spp, *Lactobacillus* spp. and *E. corrodens* and most of these bacteria showed sensitivity to these materials and in some of these studies, this antimicrobial effect was satisfactory in comparison with Chlorhexidine and not very different from the positive controls they used; these results are in line with our study. Van der Weijden et al. (1998) and Sher et al. (2011) in the separate studies reported that their mixed herbal extract mouthwash showed a weak antibacterial effect against oral bacteria on their experimental and dental plaque model study, respectively, that is against our result and the different results is probably because of different conditions of the mouth and *in vitro* media. Extract diluting in saliva content that can change the essence of these extracts, possible absorbance of the material by oral mucosa or dental tissue, pH of the saliva and its effects on the function of bacteria and extracts, the amount of CO_2 in the mouth, interference of several bacteria to induce gingival disease and different bacterial resistance on the oral context are all factors that makes some difficulties to compare the results of these studies with other *in vitro* studies.

It should be pointed out that in none of the studies in our literature, the antibacterial activity of herbal extracts or mouthwashes was assayed against *L. casei* subsp. casei and the studies worked on *L. fermentum* and *E. corrodens* and *A. viscosus* in aerobic conditions were very rare. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Parekh and Chanda, 2007). The traditional healers use water primarily as the solvent, but Parekh and Chanda (2007) found that plant extracts prepared with methanol and ethanol as solvents provided more consistent antimicrobial activity and some of the studies in our literature reported that the ethanolic extract was found to be the most effective one (Abdeirahman et al., 2002; Negi et al., 2003), however, some studies demonstrated that the antimicrobial activity of the aqueous extract they used was as strong as their alcoholic extract (Muangsan et al., 2008; Rani et al., 2004), and even some studies reported better effects of their aqueous extract (Al-Bayati et al., 2008; Aqil et al., 2005).

The differences in extraction processes and solvents, antimicrobial assays and resistance of bacteria towards different drugs due to modification of the target site, by pass of pathways, decreased uptake of the antimicrobial agent, enzymatic inactivation or modification of the drug, solubility and diffusion of active compounds in agar media, beside different condition the herbal extracts face the bacteria, which can be influenced by the study design (*in vivo* vs. *in vitro*), can cause different results and reports about the efficacy of different herbal extracts.

As our study was the primary screening antibacterial activity of these extracts, assaying minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of them are suggested in order to make the results more subjective. These findings can form the basis for further phytochemical studies to isolate active compounds, elucidate the structures, evaluate them against wider range of bacterial strains, dental plaque and *in vivo* models and tested them for their safety and efficacy to find new therapeutic principles against infectious disease. Further investigations are warranted to determine whether mouth rinses and other oral preparations with antibacterial effects might be determined from these plants.

REFERENCES

Abdeirahman HF, Phil M, Skaug N, Francis GW (2002). *In vitro* antimicrobial effects of crude Miswak extracts on oral pathogens. D.

J., 14(1): 26-32.
Al latif T, Ababneh H (1995). The effect of the extract of miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria (1995). Int. Dent. J., 45: 218-122.
Al Sadhan RI, Almas K. Miswak (chewing stick): A cultural and scientific heritage (1999). SDJ., 11(2): 80-87.
Al-Bayati FA, Sulaiman KD (2008). *In vitro* antimicrobial activity of *Salvadora persica* L. extracts against some isolated oral pathogens in Iraq. Turk. J. Biol., 32: 57-62.
Alm A (2008). On dental caries and caries-related factors in children and teenagers. Swed. Dent. J. suppl. 195: 7-63.
Al-Malik MI, Rehbini YA (2006). Prevalence of dental caries, severity, and pattern in age 6 to 7-year-old children in a selected community in Saudi Arabia. J. Contemp. Dent. Pract., 7(2): 046-054.
Almas K (1993). Miswak and its role in oral health Post. Grad. Dent., 3: 214-219.
Almas k (1999). The antimicrobial effect of extracts of Azadirachtaindica (Neem) and Salvadora persica (Arak) chewing sticks. Indian. J. Dent. Res., 10(1): 23-26.
Almas K (2002). The effect of Salvadora Persica extract (Miswak) and Chlorhexidinegluconate on dentin: A SEM study. J. Contemp. Dent. Prac., 3: 027-035.
Almas K (2004). The immediate antimicrobial effect of a toothbrush and Miswak on cariogenic bacteria: A clinical study. J. Contemp. Dent. Pract., 5(1): 1-9.
Almas K, Al-Baghieh N (1999). The antimicrobial effect of bark and pulp extracts of miswak, Salvadorapersica. Biomed. Lett., 60: 71-75.
Almas K, Al-Baghieh N, Akpata E (1997). *In vitro* antimicrobial effect of extracts of freshly cut and 1-month-old miswak (chewing stick).Biomed. Lett., 56: 145-149.
Al-Sabawi NAK, Al-Sheikh Abdal AK, Taha MY (2007). The antimicrobial activity of Salvadora Persica solution (Miswak-Siwak) as root canal irrigant (A comparative study). J. Pure. Appl. Sci., 4: 69-91.
Alzoreky NS, Nakahara K (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. Int. J. Food Microbiol., 80(3): 223-230.
Aqil F, Khan MS, Owais M, Ahmad I (2005). Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant *Staphylococcus aureus*. J. Basic. Microbiol., 45(2): 106-114.
Aqil F, Ahmad I (2003). Broad-spectrum antibacterial and antifungal properties of certain traditionally used Indian medicinal plants. World. J. Microbiol. Biotechnol., 19(6): 653-657.
Bauer AW, Kirby WMM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by standardized single disc method. Am. J. Clin. Pathol., 36: 493-496.
Billaud C (2001). Composition, nutritional value and physiological properties. Adrian J. Fenugreek. Sciences-des-ailments, 21: 3-26.
Chen CK, Wilson ME (1992). *Eikenella corrodens* in human oral and non-oral infections: a review. J. Periodontal, 63(12): 941-953.
Ciancio SG (1995). Chemical agents: plaque control, calculus reduction and treatment of dentinal hypersensitivity. Periodontal, 2000, 8: 75-86.
Darout IA, Albandar JM, Skaug N (2008). Periodontal statues of adult Sudanese habitual users of miswak chewing sticks or toothbrushes. Acta. Odontol. Scand., 58(1): 25-30.
Darout IA, Albandar JM, Skaug N, Ali RW (2002). Salivary microbiota levels in relation to periodontal status, experience of caries and miswak use in Sudanese adults. J. Clinic. Perio., 295: 411-420.
Darout IA, Christy AA, Skaug N, Egeberg PK (2000). Identification and quantification of some potentially antimicrobial anionic components in Miswak extract. Ind. J. Pharmacol., 32: 11-14.
Duraipandyan V, Ayyanar M, Ignacimuthu S (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC. Complement. Altern. Med., 6: 35
Enne VI, Livermore DM, Stephens P, Hall LMC (2001). Persistence of sulphonamid resistance in *Escherichia coli* in the UK despite national prescribing restriction.Lancet. 28: 357(9265): 1325-1328.
Ezmirly ST, Chen JC, Wilson SR (1981).Isolation of glucotropaeolin from *Salvadora persica*. J. Chem. Soc. Pak., 3: 9-12.
Fernandez MA, Garcia MD, Saenz MT (1996). Antibacterial activity of the phenolic acids fractions of *Scrophularia frutescens* and *Scrophularia sambucifolia*. J. Ethnopharmacol., 53(1): 11-14.
Goldstein Ellie JC, TarenziLia A, Agyare EO, Berger JR (1983). Prevalence of Eikenellacorrodens in dental plaque. J. Clin. Microbiol., 17(4): 636-639.
Groppo FC, Bergamaschi CC, Cogon K, Franz-Montana M, Motta RHL, Andrade ED (2008). Use of phototherapy in dentistry: A review article. Phytother. Res., 22: 993-998.
Haghighati F, Jaafari S. BeytElahi JM (2003). Comparison of antimicrobial effects of ten herbal extracts with chlorhexidine on three different oral pathogens; an *in vitro* study. HAKIM, 6: 71-76.
Hirasawa M, Shouji N (1999). Three kinds of antibacterial substances from Lentinusedodes (Berk) Sing. (Shiitake, an edible mushroom). Int. J. Antimicrob. Agents, 11: 151-157.
Iranian Herbal Pharmacopeia. Isfand 1381. Proj: 14088.
Jacquelin LF, Brisset L, Lemagrex E, Carquin J, Gelle MP, Choisy C (1995). Prevention of cariogenic dental plaque. Study of the structures implicated in the Streptococcus mutans and Streptococcus sobrinus adhesion and coaggregation. Pathol. Biol., 43: 371- 379.
Kakiuchi N, Hattori M, Nishizawa M (1986). Studies on dental caries prevention by traditional medicines. Inhibitory effect of various tannins on glucan synthesis by glycosyltransferase from *Streptococcus mutans*. Chem. Pharm. Bull., 34: 720-725.
Kartal M, Yıldız S, Kaya S, Kurucu S, Topçu G (2003). Antimicrobial activity of propolis samples from two different regions of Anatolia. J. Ethnopharmacol., 86(1): 69-73.
Katsura H, Tsukiyama R, Suzuki A, Kobayashi M (2001). *In vitro* Antimicrobial Activities of Bakuchiol against Oral Microorganisms. Antimicrob. Agents. Chemother., 45: 3009-3013.
Koo H, Gomes BPFA, Rosalen PL, Ambrosano GMB, Park YK, Cury JA (2000). *In vitro* antimicrobial activity of propolis and Arnica Montana against oral pathogens. Arch. Oral. Biol., 45(2): 141-148.
Lee Sean S, Zhang Wu, Yiming Li (2004). The antimicrobial potential of 14 natural herbal dentifrices. J. Am. Dent. Assoc., 135: 1133-1141.
Lindhe J, Thornily K, Nicklaus P (2008). Clinical periodontology and implant dentistry. 5th Ed. Willey Blackwell, Munksgaard Copenhagen, pp. 106-116.
Loesche WJ (1996). Microbiology of dental decay and periodontal disease. In: Baron's Med. Microbiol. 4th Ed., University of Texas Medical Branch.
Machado Thelma de B, Leal Ivana CR, Amaral ACF, Santos KRN, Silva MG, Kuster RM (2002). Antimicrobial ellagitannin of *Punica granatum* fruits. J. Braz. Chem. Soc., 13(5): 606-610.
Manson JO, Elley BM (2000). Outline of periodontics, 4th Reed Elsevier, pp. 381-385.
Marchese A, Schito GC (2001). Resistance pattern of lower respiratory tract pathogens in Europe. Int. J. Antimicrob. Agents. 16 Suppl., 1: S25-2S9.
Marsh PD (1992). Microbiological aspects of the chemical control of plaque and gingivitis. J. Dent. Res., 71: 1431-1438.
Marsh PD (1994). Microbial ecology of dental plaque and its significance in health and disease. Adv. Dent. Res., 8(2): 263-271.
Mehrabian S, Majd A, Majd I (2000). Antimicrobial effects of three plants (rubiatinctorum, carthamustinctorius and juglansregia) on some airborne microorganisms. Aerobiologia., 16(3): 455-458.
Meléndeza PA, Capriles VA (2006). Antibacterial properties of tropical plants from Puerto Rico. Phytomed., 13(4): 272-276.
Menezes SM, Cordeiro LN, Viana GS (2006). Punicagranatum (Pomegranate) Extract is active against dental plaque. J. Herb. Pharmacother., 6(2): 79-92.
Menezes SM, Cordeiro LN, Viana GS (2006). Punicagranatum (pomegranate) extract is active against dental plaque. J. Herb. Pharmacother., 6: 79-92.
Mothana RAA, Abdu SAA, Hassan S, Althaeas FMN, Alaghbari SAZ, Lindequist U (2008). Antimicrobial, antioxidant and cytotoxic activities and phytochemical screening of some Yemeni medicinal plants. Evid. Based. Complement. Alternat. Med., pp. 1-8.
Muangsan N, Senamontee V (2008). Antimicrobial Effects of Some Medicinal Plant Extracts Against Bacteria Associated with Black Disease. ISHS Acta Horticulturae. Int. Workshop on Med. Aromatic Plants (Abstract), p. 786.
Naz S, Siddiqi R, Ahmad S, Rasool SA, Sayeed SA (2007). Antibacterial activity directed isolation of compounds from *Punica*

granatum. J. Food. Sci., 72: 341-345.
NCCLS –National committee for Clinical Laboratory Standards (2001). Performance standards for anti-microbial susceptibility testing: eleventh informational supplement. Document M100-S11, Wayne, PA, USA.
NCCLS- National committee for Clinical Laboratory Standards (2004) Performance standards for Antimicrobial susceptibility testing. Fourteenth informational supplemented. M100 - S14, Wayne PA, USA.
Negi PS, Jayaprakasha GK (2003). Antioxidant and antibacterial activities of *Punica granatum* peel extracts. J. Food. Sci., 68: 1473-1477.
Newman MG, Takei HH, Klokkevold P, Carranza FA (2006). Carranza's Clinical Periodontol., 10th Ed., 9: 134 -142.
Parekh J, Chanda S (2007). *In vitro* screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. Afr. J. Microbiol. Res., 6: 092-099.
Pereira JV, Pereira MSV, Sampaio FC, Sampaio MCC, Alves PM, Araçjo CRF, Higino JS (2006). *In vitro* antibacterial and anti adherence effect of *Punica granatum* Linn extract upon dental biofilm microorganisms. Braz. J. Pharmacogn., 16: 88-93.
Pereira JV, Pereira MSV, Sampaio FC, Sampaio MCC, Alves PM, Araçjo CRF, Higino JS (2006). *In vitro* antibacterial and ant adherence effect of *Punica granatum* Linn extract upon dental biofilm microorganisms. Braz. J. Pharmacogn., 16: 88-93.
Poole K (2001). Overcoming antimicrobial resistance by targeting
Poureslami HR, Makarem A, Faraz M (2007). Paraclinical effects of Miswak extract on dental plaque. D. R. J., 4(2): 106-110.
Rani P, Kullar N (2004). Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi*. Phytother. Res., 18(8): 670-673.
Rasool SN, Jaheerunnisa S, Suresh Kumar Chitta. Jayaveera KN. (2008) Antimicrobial activities of Plumeria acutifolia. J. Med. Plant Res., 2(4): 077-080.
Reddy MK, Gupta SK, Jacob MR, Khan SI, Ferreira D (2007). Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum* L. Planta. Med., 73: 461-467.
resistance mechanisms. J. Pharm. Pharmacol., 53(3): 283-284. resistance/docs/EGlobal_Strat.pdf.
Scalbert A (1991). Antimicrobial properties of tannins. Chemistry. 30(12): 3875-3883.
Schüpbach P, Osterwalder V, Guggenheim B. (1995). Human root caries. Microbial in plaque covering sound, carious and arrested carious root surfaces. Caries. Res., 29(5): 382-395.
Shahidi B (2004). Evaluation of antibacterial properties of some medicinal plants used in Iran. J. Ethnopharmacol., 94: 301-305.
Sharma RD, Sarkar A, Hazra DK (1996). Hypolipidaemic effect of fenugreek seeds: a chronic study in non-insulin dependent diabetic patient. Phytother. Res., 10: 332–334.
Sher H, Al-yemeni MN, Wijaya L (2011). Ethnobotanical and antibacterial potential of Salvadora persica l: A well-known medicinal plant in Arab and Unani system of medicine. J. Med. Plant. Res., 5(7): 1224-1229.
Silva M, Higino J, Pereira J, Siqueira-Jçnior J, Pereira M (2008). Antibiotic activity of the extract of *Punica granatum* Linn over bovine strains of *Staphylococcus aureus*. Rev. Bras. Farmacogn., 18(2): 209-212.
Slots J, Rams TE Slots J, Taubman MA (1992). Microbiology of periodontal disease. In: Contemporary Oral Microbiology and Immunology. Mosby Year Book, St. Louis, USA, pp. 425-443.
Sofrata AH, Caisson Rolf LK, Lingsçm PK, Gustafsson AK (2008). Strong antibacterial effect of miswak against oral microorganisms associated with periodontitis and caries. J. Periodontol., 79(8): 1474-1479.
Spacciapoli P, Buxton D, Rothstein D, Friden P (2001). Antimicrobial activity of silver nitrate against periodontal pathogens. J. Periodont. Res., 36: 108-113.
Stavri M, Mathew KT, Gibbons S (2006). Antimicrobial constituents of *Scrophularia deserti.* Phytochem., 67: 1530-1533.
Swiatek L (1970). Pharmacobotanical investigation on some Scrophulariaceae species. IV. Chemical constituents of the herb of Scrophularianodosa. Dissertations. Pharm. Pharmacol., 22: 321-328.
Takarada K, Kimizuka R, Takahashi N, Honma K, Okuda K, Kato T (2004). A comparison of the antibacterial efficacies of essential oils against oral pathogens.Oral. Microbiol. Immunol., 19(1): 61-64.
Theodore MR, Harald OH, Edward JS (2006). Sturdevanat's Art and Science of Operative Dentistry 5th Ed., pp. 75-93.
Tichy J, Novak J (1998). Extraction, Assay, and Analysis of Antimicrobials from Plants with Activity against Dental Pathogens (Streptococcus sp.). J. Altern. Compl. Med., 4(1): 39-45.
Tong SH, Yan J, Lou J (2006). Preparative isolation and purification of harpagoside from *Scrophularia ningpoensis* hemsley by high speed counter-current chromatography. Phytochem. Anal., 17(6): 406-408.
Vahabi S, Habibi S (2007). Effects of the root extracts of Malvasylvesteris and *Salvadora persica* on two oral streptococci. On line index; ISI number: H 829C0389, IDS number: BG293, ISBN: 978-88-7587-383-7: 87-94.
Van der Weijden GA, Timmer CJ (1998). The effect of herbal extracts in an experimental mouth rinse on established plaque and gingivitis. J. Clin. Periodontol., 25: 399-403.
Vasconcelos LCS, Sampaio MCC, Sampaio FC, Higino JS (2003). Use of *Punica granatum* Linn. as an antifungal agent against candidiasis associated with denture stomatitis. Mycoses, 46: 192-196.
Viola S (1966). Medicinal plants and Flora Italiana. Maestri Veleno of Milan, p. 179.
Voravuthikunchai SP, Sririrak T, Limsuwan S, Supawita T, Iida T, Honda T (2005). Inhibitory effects of active compounds from *Punica granatum* pericarp on verocytotoxin production by enterohemorrhagic *Escherichia coli* O157: H7. J. Health Sci., 51: 590-596.
WHO publication (2001). WHO global strategy for containment of antimicrobial resistance. Available on internet at: http://www.who.int/emcdocuments/antimicrobial_
Won Sick Woo (1963). Pharmacologically active component Scrophularia root, ρ-methoxycinamic acid and its anti-pyretic. Yakhak Hoeifi, 21: 55-57.