Journal of Applied Sciences

ISSN 1812-5654

Journal of Applied Sciences 11 (5): 878-882, 2011
ISSN 1812-5654 / DOI: 10.3923/jas.2011.878.882

In vitro Comparison of the Antimicrobial Activity of Ten Herbal Extracts Against *Streptococcus mutans* with Chlorhexidine

[1]Z. Dalirsani, [2]M. Aghazadeh, [3]M. Adibpour, [1]M. Amirchaghmaghi, [1]A. Pakfetrat, [1]P. Mosannen Mozaffari, [4]M. Mehdipour and [4]A. Taghavi Zenooz
[1]Dental Research Center, Mashhad University of Medical Sciences, Department of Oral Medicine, Mashhad School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran
[2]Department of Microbiology, Tabriz Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Department of Medical Parasitology of Tabriz Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
[4]Department of Oral Medicine, Tabriz Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract: The use of mouthwash has increased because of attention to oral hygiene. Herbal mouthwashes have fewer side effects and are more economic than similar chemical drugs. In this study, the antimicrobial effects of ten herbal extracts on *Streptococcus mutans* were compared with chlorhexidine. Thirty grams of ten plants including thyme, mint, garlic, cinnamon, chamomile, tea tree, clove, spearmint, sage, and rosemary were dissolved in 100 mm of pure methanol and placed on a shaker for 48 h. Then, after passing the solution through a filter, they were put in an incubator at 37 centigrade degee for 48 h. *Streptococcus mutans* was cultured on blood agar. Chlorhexidine discs were used as positive controls while methanol and blank discs were used as negative controls. After 24 h the diameters of the halos indicative of lack of growth in each disc were measured with a pair of calipers. The zone diameters around each disc were compared with chlorhexidine using T test analysis. The inhibitory zone has observed around the Rosemary extract discs. Rosemary was found as a potent antimicrobial plant. More studies are suggested for production of herbal mouthwashes.

Key words: Plant extract, *Streptococcus mutans*, inhibitory effect, antimicrobial activity, chlorhexidine

INTRODUCTION

Artificial drugs have unpleasant side effects, on the other hand, the number of drug resistant microorganisms is increasing. So researchers are trying to pay more attention to herbal drugs. Research centers and world health organization prepare lots of programs to make use of plants extracts.

Mouthwashes for example, chlorhexidine, have several adverse effects despite good plaque control and an antimicrobial effect (Gurgan *et al*., 2006; Jenabian *et al*., 2008).

Finding plants that have antimicrobial effects and using them as mouthwashes have advantages, such as, a decrease of side effects and also they are more economical.

Most studies have been done on skin, respiratory and urinary system pathogens and there has been little research about oral pathogens (Ahmad and Beg, 2001).

Researchers have evaluated antimicrobial effects of some plants with different methods. Among them, one study showed that persica mouthwash cannot alter oral microbiota especially *Streptococcus mutans*, so it cannot prevent dental caries (Jajarm *et al*., 2009).

Bozin *et al*. (2007) detected the antibacterial activity of rosemary and sage on *Escherichia coli*, *Salmonella typhi*, *S. enteritidis* and *Shigella sonei*.

De *et al*. (1999) evaluted antimicrobial activities of 35 different Indian spices traditionally used. This study indicated that clove, cinnamon, bishop's weed, chilli, horseraddish, cumin, tararind, black cumin, pomegranate

Corresponding Author: Dalirsani Zohreh, Department of Oral Medicine, School of Dentistry, Vakil Abad Street, Mashhad, Khorasan Razavi, Postal Code 91735, Mashhad, Iran
Tel: +989155002857 Fax: +985118829500

seeds, nutmeg, garlic, onion, tejpat, celery, cambodge, have potent antimicrobial activities against the test organisms such as *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae*.

Few research studies are available on antibacterial effects of traditional plants on *Streptococcus mutans*. One research revealed the crude extract *endophytic Streptomyces* sp. ST8 decrease bacterial adherence of *Streptococcus mutans* (Taechowisan *et al*., 2008).

In Xavier and Vijayalakshmi (2007) study, ethanol extracts of Allium sativum bulb and Azadirachta indica leaf extracts exhibited high degree of inhibition effect on *Streptococcus mutans* (Xavier and Vijayalakshmi, 2007) Crude aqueous extract of *Piper betle* L. exhibited reduced effect towards the growth, adhering ability, glucosyltransferase activity against *Streptococcus mutans* (Nalina and Rahim, 2007). Because this microorganism has been implicated as one of the oral bacteria that cause dental caries, this study was initiated to complete previous studies on the antimicrobial effects of some plants that are traditionally used as medicine against *Streptococcus mutans*. To the best of our knowledge, antimicrobial activity of these selected plants has not evaluated on *Streptococcus mutans*. In this study, the antimicrobial effects of ten herbal extracts on *Streptococcus mutans* were compared with chlorhexidine whose antimicrobial activity has been demonstrated in some previous studies (Gurgan *et al*., 2006; Van der Vyver *et al*., 2009). These studies could prove useful for providing herbal mouthwash with antibacterial activity against *Streptococcus mutans*.

MATERIALS AND METHODS

The test organism; Streptococcus mutans was a standard strain ATCC-1683 that was prepared from the Scientific and Industrial Research Center in Tehran, Iran in April 2008. For this test, 0.5 Mc Farland standard microbial suspension was used which contains 10^8 bacteria mL^{-1} nutrient broths.

Streptococcus mutans was cultured on blood agar which was used as a culture media. The medicinal plants traditionally used in medicines for oral and gingival disease were selected. These 10 plants were: *Matricaria chamomillathyme* (chamomile), *Mentha arvensis* (mint), *Allium vineale* (garlic), *Cinnamomum zeylanicum* (cinnamon), *Melaleuca alternifolia* (tea tree), *Eugenia aromatica* (clove), *Mentha spicata* (spearmint), *Salvia officinalis* (sage) *Rosemaryinus officinalis* (rosemary) and *Thymus serpyllum* (thyme).

For Preparation of plant extracts, the method of Alade and Irobi (1993) was adapted with little modifications. Thirty grams of each powdered plant material were dissolved in 100 mm of pure methanol and placed on a shaker for 48 h (Alade and Irobi, 1993). Each mixture was stirred every 24 h using a sterile glass rod and then was passed through a Whatman filter paper No. 1 (Whatman, UK). They were kept in an incubator at 37°C for 48 h to produce 0.5 to 2.5 mg mL^{-1} concentrates.

The antimicrobial sensitivity of test extracts was determined by the standard Disc diffusion method of Bauer *et al.* (1966).

One hundred needle tips containing 10 disks were placed on a medical vial and were sterilized with autoclave. In sterile conditions, every needle consisting of 10 disks was placed into a glass containing one of the plant methanol extracts and covered.

After an hour the discs were removed and put in a 40°C incubator for 20 min to be dried.

On each plate, one plant extract disc, one 0.2% chlorhexidine disc which was used as a positive control and one methanol and one blank disc which were used as negative controls were placed. These disks were placed at a distance of 15 mm from the edge of the plate and 24 mm from the center of the next disk. The plates were incubated at 37°C for 24 h.

After 24 h, the antimicrobial activity was evaluated by measuring the inhibitory zone diameter observed with a pair of calipers. This test was repeated 10 times for each plant extract.

The zone of inhibition of each test plant against *Streptococcus mutans* was compared with chlorhexidine using T test analysis.

RESULTS

After 24 h, in all plates, no zone indicative of the lack of growth around the methanol and the empty discs which were used as negative controls was observed. In all plates, the inhibition zone around the chlorhexidine discs which were used as positive control was observed. The mean of inhibition zone was 14.06 mm; S.D. = 1.70 mm.

Among test plants, there was a halo indicative of the lack of growth around rosemary and clove discs. The mean of the inhibitory zone related to rosemary and clove were 11.52 mm; SD = 0.96 mm and 15.26 mm; SD = 1.55 mm, respectively (Fig. 1, 2 and Table 1).

A sample from the zone of inhibition around each plant was taken. In a gram staining sample taken from the zone of inhibitory around the clove disks, *Streptococcus*

Table 1: Comparison of the mean the inhibitory zone of the plant extracts discs with chlorhexidine

Herbal extracts	The mean of inhibitory zone (average±SD)	Comparison with chlorhexidine (PV)
Garlic	0.60	p>0.05
Chamomile	0.60	p>0.05
Cinnamon	0.60	p>0.05
Clove	15.26±1.55*	0.001
Mint	0.60	p>0.05
Rosemary	11.52±0.96	0.001
Sage	0.60	p>0.05
Spearmint	0.60	p>0.05
Tea tree	0.60	p>0.05
Thyme	0.60	p>0.05
Chlorhexidine	14.06±1.70	_

*The hemolysis zone around the clove disc

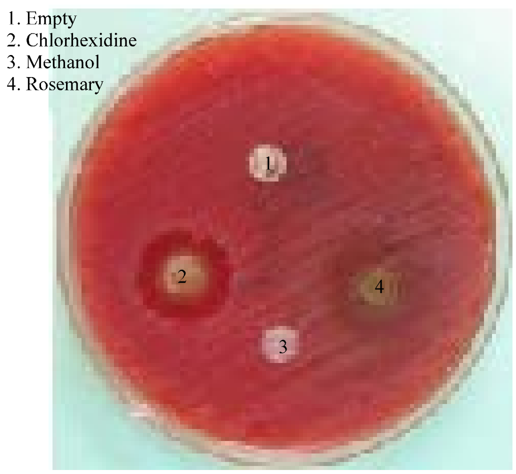

Fig. 1: The inhibitory zone around the rosemary disc

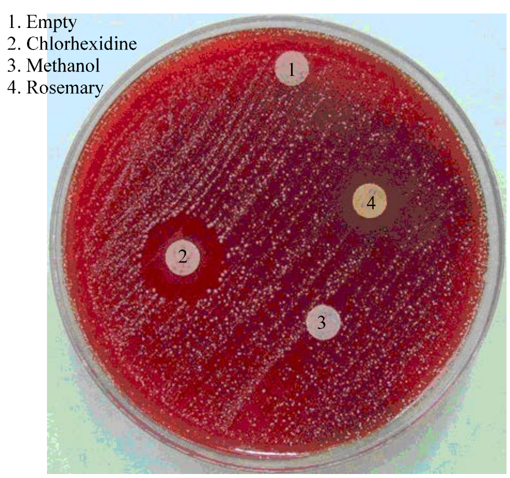

Fig. 2: The hemolysis zone around the clove disc

mutans was observed by microscope. Most probably, the acidic pH of clove led to lyses of blood agar culture media. Clove pH which was determined with chromatograph paper was found to be between 2.0 to 2.5 which demonstrate that the clove didn't have antimicrobial activity. These halos are related to the ability of clove for hemolysis. Then, this test was repeated for clove extract in empty blood agar plates in which the hemolysis zone, was also observed.

The mean of the inhibitory zone related to rosemary was 11.52±0.96 and it was significantly less than chlorhexidine (p = 0.001) (Table 1).

The result of the screening of antibacterial activity of 10 plant extracts against *Streptococcus mutans* is shown in Table 1. As the diameter of every disc was 0.6 mm, according to this table, except rosemary and clove discs, halo indicative of the lack of growth wasn't seen around other plant discs.

DISCUSSION

In this study, the antimicrobial activity of 10 traditional plant extract against *Streptococcus mutans* was evaluated by the standard Disc Diffusion method. The results of this study showed that only rosemary had antimicrobial activity against *Streptococcus mutans*. Researchers have studied the antimicrobial effects of some plant extracts but little research has been done on *Streptococcus mutans.*

These studies showed antimicrobial activity of other plants species on *Streptococcus mutans*, but other kinds of herbal extracts such as ethanol or aqueous extracts were evaluated (Nalina and Rahim, 2007).

To the best of our knowledge, antimicrobial activity of herbal extracts which are tested in present study have not evaluated against *Streptococcus mutans*. Some test plants had antimicrobial effects in previous research but in the present study, antimicrobial activity was not seen.

For example, Groppo *et al*. (2002) studied antimicrobial activity of garlic, and tea tree oil. In this study, dishes containing blood agar and Mitis Salivavius Bacitaci agar were incubated with subjects' saliva total microorganisms. The tea tree oil group showed antimicrobial activity against *Streptococcus mutans* and other oral microorganisms but the garlic group showed antimicrobial activity, just against *Streptococcus mutans* (Groppo *et al*., 2002).

In other research, the inhibitory effect of garlic was determined (Fani *et al*., 2007; Groppo *et al*., 2007).

Fani *et al*. (2007) studied inhibitory activity of the aqueous extract of garlic on multidrug-resistant strains of *Streptococcus mutans* isolated from human carious teeth. This study showed inhibitory activity of garlic *Streptococcus mutans* comparable with chlorhexidine by disc sensivity test and broth dilution methods.

Despite the antibacterial effects of garlic extract, side effects such as unpleasant taste, halitosis and nausea were reported (Groppo *et al*., 2007).

In Bozin *et al*. (2007) study the essential oils of rosemary and sage was analyzed by means of gas chromatography-mass spectrometry for their antimicrobial activity against 13 bacterial strains. Similar to present study the essential oils of rosemary and sage showed antimicrobial activity against some bacteria.

Also, Cervenka *et al*. (2006) studied the antimicrobial activity of seventeen spices and medicinal plant extracts against *Arcobacter butzleri* by disc diffusion methods. They determined cinnamon, barberry, chamomile, sage and rosemary extracts had strong antimicrobial activity toward the arcobacter strains tested. The methanol extracts of these plants, except cinnamon and rosemary, showed higher antimicrobial activity than the chloroform extracts.

In contrast to the present study, other studies showed the antimicrobial effect of some plants. The study of the effect of aromatic oils against *Pseudomonas aeruginosa* by the cup-palate methods, showed that cinnamon water possesses profound activity against *Pseudomonas aeruginosa*. But, clove, dill and peppermint waters exhibited no significant preservative actions (Ibrahim and Ogunmodede, 1991).

In another study, mixed extracts were prepared from Chinese chive, cinnamon, and corni fructus, which exhibited antimicrobial activity against *Escherichia coli*. These extracts had excellent stability to heat, pH, and storage (Mau *et al*., 2001).

Ankri and Mirelman (1999) showed that garlic allicin exhibits antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria including multidrug-resistant enterotoxic genetic strains of *Escherichia coli*. Also, garlic allicin have antifungal activity, antiparasitic activity and antiviral activity. The main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g. alcohol ehydrogenase, thioredoxin reductase, and RNA polymerase. It can affect metabolism of proteinsase involved in the virolence of *E. coli*.

Sasaki *et al*. (1999) detected the antibacterial activity of garlic and showed that the usage of fresh garlic powder was more effective than old garlic powder.

Weseler *et al*. (2005) studied the antibacterial activity of chamomile against *Helicobacter pylori*; chamomile inhibited bacterial growth. Another study demonstrated that chamomile has moderate antimicrobial activities (McKay and Blumberg, 2006). These results are different from present research in which the antibacterial activity of chamomile wasn't observed.

Feres *et al*. (2005) showed that the antimicrobial effects of clove and sage were less marked in comparison to chlorhexidine. This result was similar to the present results in which neither plants exhibited antibacterial activity.

In contrast, other studies proved the antimicrobial effects of these plants (Bozin *et al*., 2007; De *et al*., 1999). These different results can be due to variant bacterial strains. Also, some plants such as, thyme and sage have different strains in different countries. On the other hand, differences in extract type and concentration could lead to differences in results.

For example, Mahasneh and El-Oqlah (1999) studied the antimicrobial activity of petroleum ether, ethanol, butanol, and aqueous crude extracts of nine plants against four bacterial and three fungal species. Methanol and hexane extracts did not show any activity. But, the butanol extracts Ononis Spinosa (OS), Bryonia Syriaca (BS) had high moderate antifungal activity.

Ahmad *et al*. (1998) studied 82 Indian medicinal plants traditionally used in medicines, by using well dioffusion method and determined that alcoholic extracts showed greater activity than their corresponding aqueous and hexane extracts (Van der Vyver *et al*., 2009).

To the best of our knowledge, as few studies have been done on antimicrobial effects of medical plants against oral pathogens, it is better that the effect of herbal extracts on other oral bacteria that have cariogenic activity be studied. Because of the antimicrobial effects of some medical plants, which have minimal side effects in comparison with chemical drugs, more *in vivo* and *in vitro* investigations about oral cavity flora should be recommended. It is suggested that more research should be carried out to find plants with antimicrobial activity for producing herbal mouthwashes.

CONCLUSION

This study demonstrated that rosemary has antibacterial activity against *Streptococcus mutans*. If similar results are confirmed in clinical trials, this plant extracts can be used to produce new, useful and economic antimicrobial mouthwashes.

REFERENCES

Ahmad, I. and A.Z. Beg, 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharmacol., 74: 113-123.

Ahmad, I., Z. Mehamood and F. Mohammad, 1998. Screening of some Indian medicinal plants for their antimicrobial properties. J. Ethnopharmacol., 62: 183-193.

Alade, P.I. and O.N. Irobi, 1993. Antimicrobial activities of crude leaf extract of *Acalypha wilkensiana*. J. Ethanopharmacol., 39: 171-174.

Ankri, S. and D. Mirelman, 1999. Antimicrobial properties of allicin from garlic. Microbes Infect., 1: 125-129.

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

Cervenka, L., I. Peskova, E. Foltynova, M. Pejchalova, I. Brozkova and J. Vytrasova, 2006. Inhibitory effects of some spice and herbal extracts against arcobacter butzeln. Curr. Microbiol., 53: 435-439.

De, M., D.A. Krishna and A.B. Banerjee, 1999. Antimicrobial screening of some Indian spices. Phytother. Res., 13: 616-618.

Fani, M., J. Kohanteb and M. Dayaghi, 2007. Inhibitory activity of garlic (*Allium sativum*) extract on multidrug-resistant *Streptococcus mutans*. J. Indian Soc. Pedod. Prev. Dent., 25: 164-168.

Feres, M., L.C. Figueiredo, I.M. Barreto, M.H. Coelho, M.W. Araujo and S.C. Cortelli, 2005. *In vitro* antimicrobial activity of plant extracts and propolis in saliva samples of healthy and periodontally-involved subjects. J. Int. Acad. Periodontol., 7: 90-96.

Groppo, F.C., J.C. Ramacciato, R.H. Motta, P.M. Ferraresi and A. Sartoratto, 2007. Antimicrobial activity of garlic against oral streptococci. Int. J. Dent. Hyg., 5: 109-115.

Groppo, F.C., J.C. Ramacciato, R.P. Simoes, F.M. Florio and A. Sartoratto, 2002. Antimicrobial activity of garlic, tea tree oil and chlorhexidine against oral microorganisms. Int. Dent. J., 52: 433-437.

Gurgan, C.A., E. Zaim, I. Bakirsoy and E. Soykan, 2006. Short-term side effects of 0.2% alcohol-free chlorhexidine mouthrinse used as an adjunct to non-surgical periodontal treatment: A double-blind clinical study. J. Periodontol., 77: 370-384.

Ibrahim, Y.K. and M.S. Ogunmodede, 1991. Growth and survival of pseudomonas aeroginosa in some aromatic waters. Pharm. Acta Helv., 66: 286-288.

Jajarm, H.H., A. Jahanbin, N. Mokhber, S. Gooyandeh, A. Mansourian and J.M. Beitollahi, 2009. Effects of Persica mouthwash on oral microbiota of cleft lip and palate patients during fixed orthodontic treatment. J. Applied Sci., 9: 1593-1596.

Jenabian, N., M. Abedi, P. Tayebi and A.A. Moghadamnia, 2008. Local delivery of metronidazole and chlorhexidine as toothpaste in treatment of adult periodontitis. Int. J. Pharmacol., 4: 361-368.

Mahasneh, A.M. and A. El-Oqlah, 1999. Antimicrobial activity of herbal plants used in the tradition a medicine of Jordan. J. Ethmopharmacol., 64: 271-276.

Mau, J.L., C.P. Chen and P.C. Hsieh, 2001. Antimicrobial activity of extracts from Chinese chive, Cinnamon and *Corni fructus*. J. Agric. Food Chem., 49: 183-188.

McKay, D.L. and J.B. Blumberg, 2006. A review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita* L.). Phytother Res., 20: 519-530.

Nalina, T. and Z.H.A. Rahim, 2007. The crude aqueous extract of *Piper betle* L. and its antibacterial effect towards *Streptococcus mutans*. Am. J. Biotechnol. Biochem., 3: 10-15.

Sasaki, J., T. Kita, K. Ishita, H. Uchisawa and H. Matsue, 1999. Antimicrobial activity of garlic powder against E-coli. O-157. J. Nutr. Sci. Vitaminol., 45: 785-790.

Taechowisan, T., A. Sitthipanya, A. Wanbanjob and P. Tantiwachwuttikul, 2008. Inhibitory effects of endophytic *Streptomyces* sp. ST8 on the growth, adherence and glucosyltransferase of *Streptococcus mutans*. J. Boil. Sci., 8: 43-51.

Van der Vyver, P.J., F.S. Botha, D. Herbst and M. Ismail, 2009. Antimicrobial efficacy and irrigating potential of irrigation solutions using different activation methods. SADJ, 64: 58-60.

Weseler, A., H.K. Geiss, R. Saller and J. Reichling, 2005. A novel colorimetric broth microdilution method to determine the minimum inhibitory concentration (M/C) of antibiotics and essential oil against helicobacter pylori. Pharmazie, 66: 498-502.

Xavier, T.F. and P. Vijayalakshmi, 2007. Screening of antibiotic resistant inhibitors from indian traditional medicinal plants against *Streptococcus mutans*. J. Plant Sci., 2: 370-373.