

The antibacterial effect of sage extract (*Salvia officinalis*) mouthwash against *Streptococcus mutans* in dental plaque: a randomized clinical trial

Maryam Beheshti-Rouy¹, Mohadese Azarsina^{2*}, Loghman Rezaie-Soufi¹, Mohammad Yousef Alikhani³, Ghodratollah Roshanaie⁴, Samira Komaki¹

¹Department of Operative Dentistry, Faculty of Dentistry, Hamadan University of Medical Sciences, Hamadan, Iran.

²Department of Operative Dentistry, Faculty of Dentistry, International Branch of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

³Mohammad-Yousef Alikhani: Department of Microbiology, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.

⁴Ghodratollah Roshanaie: Department of Public Health, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.

Received: June 2013, Accepted: October 2014

ABSTRACT

Background and Objective: The aim of the study was to evaluate the clinical effects of a mouthwash containing Sage (*Salvia officinalis*) extracts on *Streptococcus mutans* (SM) causing dental plaque in school-aged children.

Material and Methods: A double blind clinical trial study was conducted in a dormitory on 70 girls aged 11-14 years having the same socioeconomic and oral hygiene conditions. These students were randomly divided into 2 groups; the first group (N=35) using Sage mouthwash, and the second group (N=35) using placebo mouthwash without active any ingredients. At the baseline, plaque samples obtained from the buccal surfaces of teeth were sent to laboratory to achieve SM colony count. These tests were reevaluated after 21 days of using the mouthwashes. Statistical data analysis was performed using t-student tests with $p < 0.05$ as the level of significance.

Results: Sage mouthwash significantly reduced the colony count ($P=0.001$). Average number of colonies in test group was 3900 per plaque sample at the baseline, and 300 after mouthwash application. In the control group, pre-test colony count was 4400 that was reduced to 4000; although this reduction wasn't significant.

Conclusion: The Sage mouthwash effectively reduced the number of *Streptococcus mutans* in dental plaque.

Keywords: anti-bacterial agents; dental plaque; *Salvia officinalis*; *Streptococcus mutans*;

INTRODUCTION

Dental caries is a worldwide oral disease, especially in developing countries, which form the major part of the world. Bacterial plaque is considered as an etiologic factor for caries, and oral self-care for plaque control is an essential step in the prevention from caries (1). *Streptococcus mutans* (SM) is the main bacteria in dental plaque, responsible for caries

*Corresponding author: Dr. Mohadese Azarsina

Address: Department of Operative Dentistry, Faculty of Dentistry, International Branch of Shahid Beheshti University of Medical Sciences, South Jamalzade Street, Tehran, Iran.
Tel: +98-21-66917171
Fax: +986134433715
E-mail: azarsina2012@yahoo.com, beheshtirouym@yahoo.com

process (2).

Due to the difficulties for teens in achieving complete plaque control, the administration of some antiplaque agents such as chemical or herbal antimicrobial dental products was suggested as an auxiliary protocol to tooth brushing (3). Considering all the disadvantages of using different chemical agents, many studies are being conducted on the effectiveness of herbal materials (4).

Recently, antimicrobial effect of sage extract has been shown experimentally (5, 6). Dry sage leaves were used in folk medicine for a variety of disorders (7). Today, sage is also used as a traditional remedy for many diseases (8, 9).

According to the results of previous studies (10, 11), and considering lack of randomized controlled trials on the effectiveness of sage extract on oral microorganisms, the aim of the present study was to evaluate the clinical effectiveness of a mouthwash containing Sage (1% *Salvia officinalis*) extract on reduction of SM in dental plaque in a group of school-aged children.

MATERIALS AND METHODS

Enrollment. A double blind randomized clinical trial was conducted among female 11-14 year-old school children of Hamadan, Iran during the year 2012. Prior to the study, ethical clearance was obtained from Hamadan University Research Ethics Board (Protocol No: 1010/9/35/16). Permission to conduct the study among the school children was obtained from their guardians. The IRCT number is 2012070710204N. Each subject was provided with a written informed consent and all of the researchers undertook Helsinki treaty.

Mouth rinse preparation. Sage mouthwash was extracted from the plant *Salvia officinalis* in the laboratory of a pharmaceutical company (Jahanghir, Tehran) by an expert pharmacologist. Leaves of the plant were chopped, fragmented, and broken into small pieces, and each 50 g of leaves were soaked in 1500 ml of solvent (50% water/ 50%ethanol [96%]) in a shaker apparatus (Heidolph Unimax; Schwabach, Germany) at 90 rpm for 48 hours. Thereafter, the solution was passed through a strainer and transferred to a rotary evaporator apparatus (Heidolph WD2000; Schwabach, Germany) to

separate the solvent from the extract. The 5% Sage mouthwash was prepared (0.5 g of extract in 100 ml distilled water) and poured into bottles each containing 240 ml of the solution. Normal saline mouthwash was prepared in the bottles with the same shape and color, to be used as control.

Selection and allocation of subjects. Sample size was determined using Biometrika table for proportions, which is based on three factors: Power of the study, Level of Significance, and the Efficacy values in the previous studies. Based on this estimation, 35 subjects were included in each group. Two stage random sampling was done to select the subjects. In the first stage, all the subjects were screened for inclusion criteria (11-14 year-old girls under the supervision of a welfare organization with same socioeconomic and nutritional conditions). Children with systemic physical or mental problems or using antibiotics within the past 1 month were excluded from the study. Children were randomly allocated to study and control groups.

Rinsing procedure. Prior to the study, the children were demonstrated the rinsing procedure. The study procedure was carried out in the school premises. The mouth rinse bottles given to the participants were unlabeled. The participants were instructed to continue their usual oral hygiene measures and not to use any other mouth rinse for the duration of the study. The subjects were demonstrated to use the mouth wash for 60 seconds, twice daily (once taken at night just before the bed time) over the 3-week study period. The participants' compliance was evaluated by measuring the remaining volume of the mouth wash that they brought back during their recalls. They were also asked to report any adverse reactions experienced during the use of their mouth wash.

Plaque sample collection. Baseline plaque samples were collected. The subjects were informed not to brush 24 hours prior to plaque collection. Plaque collection was done in the morning. Plaque samples were collected using sterile disposable sticks from the buccal surface of anterior teeth. The plaque was placed in a vial containing a transport medium and transported to 1ml Brain-Heart Infusion (BHI) [BHI; Difco, Sparks, MD, USA] culture medium. Afterwards, the samples were cultured in MSB

specific medium (A.L. Norway) containing 0.2 units per milliliter Bacitracin. The numbers of the SM colonies grown in Bacitracin culture medium were counted visually. Data were statistically analyzed by t-student test, using SPSS software (Version 16, SPSS Inc., Chicago, USA). Level of significance was set at 0.05.

RESULTS

The mean colony count scores of the study and control group, before and after mouth wash application are presented in Tables 1 and 2.

A significant difference was observed in post treatment SM counts between the study and control group (P = 0.00) and also between baseline and post treatment samples in the study group (P = 0.001). Although, no significant difference was observed in baseline SM counts between study and control group

(P = 0.65), and between baseline and post treatment in control group (P = 0.11) despite the drastic colony count reduction (Tables 3 and 4).

DISCUSSION

The present randomized controlled clinical trial was conducted to determine the effect of mouth rinse formulated from Sage extract on dental plaque SM counts among 11 -14 year-old children in Hamadan, Iran.

Salvia officinalis is one of the most commonly used herbs in traditional medicine (9, 11, 12). It has been popularly referred to as "Sage". It has been reported that sage exerts a range of therapeutic activities including antibacterial, antiviral, antifungal, and antioxidant effects (13-15). It would be of interest to determine if such an herb could also have a beneficial effect on oral health.

Table 1. Means of colony counts of 11-14 year-old children, before and after using test mouth rinse

| Index | Colony-pre | Colony-post |
|-------|------------|-------------|
| N | 35 | 35 |
| Mean | 3900 | 600 |
| SD | 1465.7 | 665.1 |

Table 2. Means of colony counts of 11-14 year-old children, before and after using placebo mouth rinse

| Index | Colony-pre | Colony-post |
|-------|------------|-------------|
| N | 35 | 35 |
| Mean | 4071.4 | 3174.3 |
| SD | 1630.7 | 1628.3 |

Table 3. Multiple comparisons of the Colony count test among the study and control group.

| Group | p.value | t | N | SD | Mean | index |
|---------|---------|------|----|--------|--------|-------------|
| study | 0.001 | 13.4 | 35 | 1465.7 | 3900 | Colony-pre |
| | | | 35 | 665.1 | 600 | Colony-post |
| control | 0.11 | 1.63 | 35 | 1630.7 | 4071.4 | Colony-pre |
| | | | 35 | 1628.3 | 3714.3 | Colony-post |

Table 4. Multiple comparisons of the Colony count test before and after using test or control mouth rinse.

| Group | p.value | T | N | SD | Mean | Index |
|-------------|---------|-------|----|--------|---------|---------|
| Colony-pre | 0.65 | 0.46 | 35 | 1630.7 | 4071.43 | control |
| | | | 35 | 1465.7 | 3900 | study |
| Colony-post | 0.00 | 10.48 | 35 | 1628.3 | 3714.29 | control |
| | | | 35 | 665.1 | 600 | study |

Several herbs have been studied for their effect on oral health (16, 17). Studies on the antimicrobial potential of the *Salvia* genus reveal a broad variability, depending on the sensitivity of microorganisms and the efficiency of the tested compounds. *Salvia* species rich in essential oils (such as *S. officinalis*) with volatile monoterpenoid as their major constituents are reported to be effective antibacterial (18).

Generally, Gram-positive bacteria are more sensitive to sage essential oil compared to other kinds of bacteria (19). SM is an anaerobic, Gram-positive bacterium with the ability to metabolize sucrose and release lactic acid. This acidic environment predisposes the enamel of the tooth to caries (20). The sensitivity of bacteria is related to the morphological structure and chemical composition of their membrane (21).

Essential oils can inhibit microorganisms by various mechanisms, in part due to their hydrophobicity. They get partitioned into the lipid bi layer of the cell membrane, making it more permeable, causing leakage of vital cell contents (22). The loss of the differential permeability character of the cytoplasmic membrane is the cause of cell death (22, 23).

The subjects of the present study were children in the age group of 11-14 years old. The prevalence of caries is relatively more in this age group (24). The microbial flora in younger children varies during mixed dentition stage (25). A pilot study was performed to determine the maximum time up to which children could rinse without any discomfort. It was observed that children could rinse up to 60 seconds.

Sage mouth rinse can be used as an adjunct for conventional methods of plaque control against dental caries. Although chlorhexidine has a proven role in reducing plaque accumulation, tooth staining is the major limiting factor for its daily use (26). Further studies need to be conducted comparing the effect of sage mouth rinse to gold standard mouth rinses.

Considering the limitations of the present study, it was concluded that sage extract mouth rinse exerted antibacterial action against *Streptococcus mutans* in dental plaque.

REFERENCE

1. Axelsson P, Nyström B, Lindhe J. The long-term effect of a plaque control program on tooth mortality, caries and periodontal disease in adults. Results after 30 years of maintenance. *J Clin Periodontol* 2004;31:749-757.
2. Matalon S, Weiss EI, Gorfil C, Noy D, Slutzky H. In vitro antibacterial evaluation of flowable restorative materials. *Quintessence Int* 2009;40:327-332.
3. Allaker RP, Douglas CW. Novel anti-microbial therapies for dental plaque-related diseases. *Int J Antimicrob Agents* 2009;33:8-13.
4. Baradari AG, Khezri HD, Arabi S. Comparison of antibacterial effects of oral rinses chlorhexidine and herbal mouth wash in patients admitted to intensive care unit. *Bratisl Lek Listy* 2012;113:556-560.
5. Škrovánková S, Mišurcová L, Machů L. Antioxidant activity and protecting health effects of common medicinal plants. *Adv Food Nutr Res* 2012;67:75-139.
6. Lixandru BE, Drăcea NO, Dragomirescu CC, Drăgulescu EC, Coldea IL, Anton L, et al. Antimicrobial activity of plant essential oils against bacterial and fungal species involved in food poisoning and/or food decay. *Roum Arch Microbiol Immunol* 2010;69:224-230.
7. Kianbakht S, Abasi B, Perham M, Hashem Dabaghian F. Antihyperlipidemic effects of *Salvia officinalis* L. leaf extract in patients with hyperlipidemia: a randomized double-blind placebo-controlled clinical trial. *Phytother Res* 2011;25:1849-1853.
8. Keshavarz M, Mostafaie A, Mansouri K, Bidmeshkipour A, Motlagh HR, Parvaneh S. In vitro and ex vivo antiangiogenic activity of *Salvia officinalis*. *Phytother Res* 2010;24:1526-1531.
9. Rodrigues MR, Kanazawa LK, das Neves TL, da Silva CF, Horst H, Pizzolatti MG, et al. Antinociceptive and anti-inflammatory potential of extract and isolated compounds from the leaves of *Salvia officinalis* in mice. *J Ethnopharmacol* 2012 ;139:519-526.
10. Haffajee AD, Yaskell T, Socransky SS. Antimicrobial effectiveness of an herbal mouthrinse compared with an essential oil and a chlorhexidine mouthrinse. *J Am Dent Assoc* 2008;139:606-611.
11. Bouajaj S, Benyamna A, Bouamama H, Romane A, Falconieri D, Piras A, et al. Antibacterial, allelopathic and antioxidant activities of essential oil of *Salvia officinalis* L. growing wild in the Atlas Mountains of Morocco. *Nat Prod Res* 2013; 27:1673-6
12. Russo P, Frustaci A, Del Bufalo A, Fini M, Cesario A. From traditional European medicine to discovery of new drug candidates for the treatment of dementia and Alzheimer's disease: acetylcholinesterase inhibitors. *Curr Med Chem* 2013;20:976-983.
13. Samuels N, Grbic JT, Saffer AJ, Wexler ID, Williams RC. Effect of an herbal mouth rinse in preventing periodontal inflammation in an experimental gingivitis model: a pilot study. *Compend Contin Educ Dent*

- 2012;33:204-6, 208-211.
14. Geuenich S, Goffinet C, Venzke S, Nolkemper S, Baumann I, Plinkert P, et al. Aqueous extracts from peppermint, sage and lemon balm leaves display potent anti-HIV-1 activity by increasing the virion density. *Retrovirology* 2008 20;5:27.
 15. Bozin B, Mimica-Dukic N, Samojlik I, Jovin E. Antimicrobial and antioxidant properties of rosemary and sage (*Rosmarinus officinalis* L. and *Salvia officinalis* L., Lamiaceae) essential oils. *J Agric Food Chem* 2007;55(19):7879-7885.
 16. Jebashree HS, Kingsley SJ, Sathish ES, Devapriya D. Antimicrobial activity of few medicinal plants against clinically isolated human cariogenic pathogens-an *in vitro* study. *ISRN Dent* 2011;2011:541421.
 17. Jayashankar S, Panagoda GJ, Amaratunga EA, Perera K, Rajapakse PS. A randomised double-blind placebo-controlled study on the effects of a herbal toothpaste on gingival bleeding, oral hygiene and microbial variables. *Ceylon Med J* 2011;56:5-9.
 18. Nadir M, Rasheed M, Sherwani SK, Kazmi SU, Ahmad VU. Chemical and antimicrobial studies on the essential oil from *Salvia santolinifolia* Boiss. *Pak J Pharm Sci* 2013;26:39-52.
 19. Kamatou GP, Viljoen AM, Gono-Bwalya AB, van Zyl RL, van Vuuren SF, Lourens AC, et al. The *in vitro* pharmacological activities and a chemical investigation of three South African *Salvia* species. *J Ethnopharmacol* 2005 1;102:382-390.
 20. Cross SE, Kreth J, Wali RP, Sullivan R, Shi W, Gimzewski JK. Evaluation of bacteria-induced enamel demineralization using optical profilometry. *Dent Mater* 2009;25:1517-1526.
 21. Horiuchi K, Shiota S, Hatano T, Yoshida T, Kuroda T, Tsuchiya T. Antimicrobial activity of oleanolic acid from *Salvia officinalis* and related compounds on vancomycin-resistant enterococci (VRE). *Biol Pharm Bull* 2007;30:1147-1149.
 22. Burt S. Essential oils: their antibacterial properties and potential applications in foods--a review. *Int J Food Microbiol* 2004 1;94:223-253.
 23. Generalić I, Skroza D, Surjak J, Možina SS, Ljubenković I, Katalinić A, et al. Seasonal variations of phenolic compounds and biological properties in sage (*Salvia officinalis* L.). *Chem Biodivers* 2012;9:441-457.
 24. Axelsson P. The effect of a needs-related caries preventive program in children and young adults - results after 20 years. *BMC Oral Health* 2006 15;6 Suppl 1:S7.
 25. Kamma JJ, Diamanti-Kipiōti A, Nakou M, Mitsis FJ. Profile of subgingival microbiota in children with mixed dentition. *Oral Microbiol Immunol* 2000;15:103-111.
 26. Varoni E, Tarce M, Lodi G, Carrassi A. Chlorhexidine (CHX) in dentistry: state of the art. *Minerva Stomatol* 2012;61:399-419.