Antibacterial efficiency of *salvia officinalis* extracts and their effect on growth, adherence and acid production of oral Mutans Streptococci

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ABSTRACT

Background: The use of antimicrobial agent to control plaque and oral disease has been advocated for a number of years. Different compounds have been delivered through mouth rinses or tooth pastes or by topical application. The purpose of this research is to find out and to compare between the anticariogenic properties of aqueous and alcoholic sage extract on the most causative cariogenic bacteria in the oral cavity (Mutans streptococci).

Materials and methods: In the present study Mutans streptococci were isolated from saliva often dental students (age range between 21-23 yrs) .These bacteria were isolated, purified and diagnosed according to morphological characteristic and biochemical tests.

Results: Agar diffusion technique showed that sage extracts (aqueous and alcoholic) were inhibited the growth of Mutans Streptococci, and the diameter of inhibition zone increased as the concentration of sage extract increased, but the effect of aqueous extract was less than the effect of alcoholic extract. The minimum bactericidal concentration of aqueous and alcoholic sage extract were 50%, 20% respectively. Also the alcoholic extract was high significant inhibit ($P<0.01$) the viable count of Mutans Streptococci in vitro in comparison to aqueous extract.

Conclusion: Alcoholic sage extract was interfered with acid production and adherence of Mutans Streptococci higher than aqueous extract resultant in reducing of acid production and inhibition of the adherence of this cariogenic bacteria; alcoholic sage extract have substantively phenomenon similar to those in chlorohexidine in comparison to aqueous extract.

Key words: Sage leaves, extracts, oral mutans streptococci. (J Bagh Coll Dentistry 2012; 24(sp. Issue 1):153-157).

INTRODUCTION

Dental caries is a multifactorial disease, which depends upon the inter-relation of four main groups of factors; dental plaque (microorganisms), carbohydrates (substrates); susceptible teeth (host) and the time factor [(1)]. The microorganisms specifically adapted for life on teeth form a film called dental plaque on the tooth surface which is a complex mass containing about 10^9 bacteria/g embedded in a polysaccharide matrix, they must have the ability to produce acid by fermentation of carbohydrate and result in demineralization of the mineral portion of teeth hard tissues followed by disintegration of the organic matrix [(2)].

The use of antimicrobial agent to control plaque and oral disease has been advocated for a number of years. Different compounds have been delivered through mouth rinses or tooth pastes or by topical application. Some chemical agents have proven to be helpful against plaque accumulation and thereby to some extent also against caries [(3)].

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Salvia officinalis (Garden sage, Common sage) is a small perennial evergreen subshrub. It is a member of the family Lamiaceae and is native to the Mediterranean region, though it has naturalized in many places throughout the world. It has a long history of medicinal and culinary use, and in modern times as an ornamental garden plant. It can be applied to external wounds; the essential oil, heated in a vaporizer, will disinfect sick-rooms. The phenolic acids in Salvia are particularly potent against *Staphylococcus aureus*. In vitro, sage oil has been shown to be effective against both gram-positive and Gram-negative bacteria including *Escherichia coli* and *Salmonella* species, and against filamentous fungi and yeasts such as *Candida albicans* [(4)]. Salvia also has an astringent action due to its relatively high tannin content and can be used in the treatment of infantile diarrhea; its antiseptic action is of value where there is intestinal infection. Rosmarinic acid contributes to the herb's anti-inflammatory activity [(5)]. Its bitter component stimulates upper digestive secretions, intestinal mobility, bile flow, and pancreatic function, while the volatile oil has a carminative and stimulating effect on the digestion. The thujone has a vermifuge action[(6)]. It is effective in reducing milk production, and can be used during the process of weaning an

infant off the breast; Salvia also deals effectively with throat infections, dental abscesses, infected gums and mouth ulcers [5].

MATERIALS AND METHODS

Stimulated saliva samples were collected under standard conditions to obtain 50 microbial samples. Dental students with no medical history aged 21-23 years were selected to participate in this study. Ten-fold serial dilutions were prepared using sterile normal saline. Two dilutions were selected and inoculated on Mitis-Salivarius Bacitracin Agar (MSB Agar), then incubated anaerobically by using a gas pack supplied in an anaerobic jar for 48 hrs at 37°C followed by aerobic incubation for 24hrs at 37°C.

A single colony from MS, was transferred to 10 ml sterile BHI-B and then incubated for 24 hrs aerobically at 37°C to activate the inoculums.

All the isolates were gram positive (Fig. 1). The motility of all types of microbial cells was examined under microscope by direct smear and without staining; the isolates were non- motile and catalase negative. Cystine Trypticase-mannitol media had been used to test the ability of MS to ferment the mannitol.

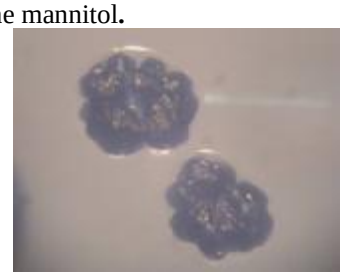

Figure 1: MS colonies on MSB agar(20x magnification).

100gm of dried leaves of Sage was infused in 500ml of boiling distilled water and left to cool to room temperature to prepare the aqueous extract[7] and 100gm of dried leaves of Sage was infused in 500ml of 98% of ethanol alcohol to prepare the alcoholic extract[8]. Agitation of the infusions with magnetic stirrer had been done alternatively; the infusion was filtered by filter paper (Wattman No.1) and the residue discarded. The extract left to dry in a Petridish at room temperature, the resulted powder kept in tightly closed glass container in refrigerator until used to prepare different concentrations.

From aqueous and alcoholic extracts of sage leaves different concentrations were prepared by using deionized sterile distilled water (for the aqueous extract) and Dimethyl formamide (DMF) for the alcoholic extract; sage leaves extract concentrations were used as follows: 50mg%, 60% and 70%for aqueous extract and 20%, 30%, 40% and 50% for alcoholic extract. Agar diffusion technique was applied to study the antimicrobial effects of both types of sage leaves extracts and CHX against the isolates spreaded on Brain Heart Infusion Agar (BHI-A); wells of equal sizes and depths were prepared in the agar using Kork porer. Single control well filled with DMF to evaluate its antimicrobial effect alone was made in each plate. Each well was filled with 50µl of a concentration was prepared from the stocks of the extracts. Inhibition zones diameters were measured using a scientific ruler; resistance of the isolates to the tested agents was indicated when there were no zones of inhibition. The diameter of inhibition zone created by the DMF was zero mm. To determine the minimum bactericidal concentrations (MBC) for the extracts, final concentrations of 20, 25, 30, 40, 50, 60 and 70%, and absolute DMF were prepared and incorporate in the BHI-A from sage leaves extracts to get 25ml of agar and sage extract then poured into Petri dishes and allowed to harden and inoculated with 0.1ml from the activated isolates of MS[9].

All these Petri dishes were incubated for 24 hrs at 37°C including the control bottles (negative control which contained BHI-A with microbial inoculums without the addition of the extract and the positive control plates which contained BHI-A and different concentrations of aqueous and alcoholic extracts of sage leaves separately without microbial inoculums). Each petridishe was checked and examined for microbial growth. The MBC was determined as the lowest concentration of sage leaves extract killed the microorganisms.

The effect of MBC and 1/2MBC of Sage aqueous extract on the growth of M.S had been tested in comparison to the control. thirty isolates of M.S were used in this experiment; after carrying out initial count for viable bacteria on MSA, 1 ml bacterial suspension inoculated into their tubes which contained full concentration MBC, 1/2MBC and the third tube as a control contained bacteria only. The three tubes already contain 10 ml of BHIB. After incubation for 18-24 hours at 37◦c. From the above tubes, a serial dilution of (10^{-1}, 10^{-2}, 10^{-4}) respectively prepared. 0.1 ml from dilution 10^{-2} & 10^{-4} to be cultivated onto the previously prepared MSB agar plates & incubated aerobically for 48 hours at 37◦c.

The adherence of M.S on the tooth surfaces was tested in laboratory according to Balekjian *et al.*[10] , in the presence of aqueous and alcoholic extracts of Sage in sucrose broth media. Sound first premolars were collected, cleaned and polished using slow speed hand piece and non fluoridated pumice then cleaned using deionized distilled water and autoclaved.

Final concentrations of 10%, 20% of Sage alcoholic extract and 25%, 50% of Sage aqueous extract were obtained in the 5ml. of the sucrose broth; the sterilized teeth were placed in the screw capped bottles, one tooth in each bottle,

Dry weight of dental plaque = weight of plaque mass on the tooth – the weight of the tooth alone.

Effects of Sage extracts (alcoholic and aqueous) on acidogenicity of M.S isolated (in vitro) was tested according to Maltz-Turkienicz *et al.*[11] Sage extracts: 10%, 20% alcoholic and 25%, 50% aqueous in sterilized CTA media. Calculation of the ΔpH: After incubation, the pH of all bottles were calculated by pH meter. ΔpH was determined according to Al-Mizraqchi [12] as follows: ΔpH = pH before incubation - pH after incubation.

The ability of adsorption of Sage aqueous and alcoholic extract to the tooth surface had been tested in comparison to CHX glucconate was according to Al-Mizraqchi [12].

RESULTS

Diameters of inhibition zones for aqueous and alcoholic extracts of sage leaves were found to be increased as the concentration of the extracts increased. Figure 2 illustrates the mean diameters of the inhibition zones in relation to the concentrations of the aqueous extract and CHX. Student's t-test showed highly significant differences among different concentrations of sage extract and CHX 0.2% . Figure 3 describes the antimicrobial effect of the alcoholic extract against M.S..

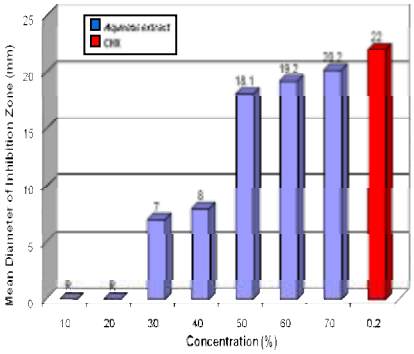

Figure 2: Comparison between the mean diameters of inhibition zones of sage aqueous extract and C

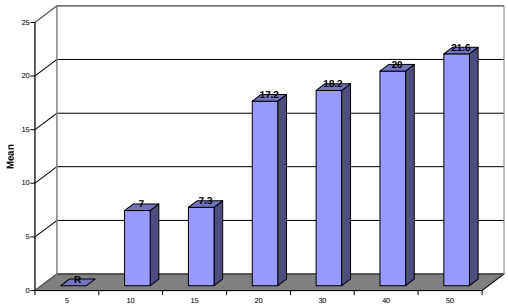

Figure 3: Comparison between the mean diameters of inhibition zones of Sage alcoholic extract.

The comparison between the anti-microbial effect of different concentrations of the aqueous and the alcoholic extract of sage showed the significance in difference between different concentrations (Tab 1).

Table 1: Statistical analysis (t-test) of the comparison between the Alcohol extract & Aqueous extract.

t-test	P-value	Sig
1.950	0.049	Sig

P<0.05 Significant

MBC of sage aqueous extract of MS (Tab. 2) was 25-50% where most isolates were inhibited by concentration equal to 50% of aqueous extract, while about 85% of the isolates were inhibited by 20% alcoholic extract.

Table 2: Minimum Bactericidal Concentrations (MBC) of Sage aqueous and alcoholic extract for Mutans Streptococci.

Type of Sage extract	Concentration of sage extract (%)										
	No. of isolates	10	20	25	30	35	40	45	50	60	70
Aqueous extract	30			2	2	3	3	4	16		
Alcoholic extract	30	5	25								

Table 3 express the compression of MS count in the presence of sage aqueous and alcoholic extracts. High significant difference between the two extracts concentrations (P-value<0.01)

Table 3: Student's t-test for the Comparison between the Effect Alcoholic and Aqueous Extract of Sage on the growth of MS (*in vitro*).

	t-test	P-value	Sig
1/2MBC	31.4	P<0.01	HS
MBc	12.2	P<0.01	HS

Statistical analysis for the mean of dry weight of plaque mass (mg) formed by Mutans streptococci after treatment with sage alcoholic and aqueous extract (*in vitro*) showed reduction in the plaque mass accumulation (Table 4).

Table 4: Statistical analysis for the mean of dry weight of plaque mass (mg) formed by Mutans streptococci after treatment with sage alcoholic and aqueous extract (*in vitro*).

	Mean dry weight (mg) of plaque mass after treatment with Sage extract			
	MBC	1/2MBC	t-test	Pvalue
Aqueous	11 $\pm$ 1.42	18 $\pm$ 1.428	22.45	P<0.01 HS
Alcoholic	8 $\pm$ 1.43	13 $\pm$ 1.80	14.14	P<0.01 HS
Control	24 $\pm$ 1.428	24 $\pm$ 1.428	-	-
t-test	10.64	16.36	-	-
P-value	P<0.01 HS	P<0.01 HS	-	-

The experiment showed the effect of 1/2 MBC and MBC (10-20%) alcoholic extract and (25-50%) aqueous extract in reducing acid production (Table5). The values of ΔpH; p-value<0.01 Highly Significant with control.

Table 5: Statistical analysis of the mean of ΔpH from acid production by Mutans Streptococci strains isolated after treatment with Sage Alcoholic and Aqueous extract.

	Mean ΔpH of Mutans streptococci after treatment with Sage extract			
	MBc	**1/2MBc**	**t-test**	**P-value**
Aqueous	1.0 ± 0.132	1.4 ± 0.141	77.71	P<0.01 HS
Alcoholic	0.5 ± 0.135	1.2 ± 0.127	26.57	P<0.01 HS
Control	2.5 ± 0.129	2.5 ± 0.129	-	-
t-test	18.44	8.682	-	-
P-value	P<0.01 HS	P<0.01 HS	-	-

*** initial pH=7.**

The results of this experiment showed that alcoholic extract of Sage have ability adsorbed to tooth surface and then release from the retention site (substantivity). This appear as inhibition zone around the tooth treated with the extract due to the release of the extract and diffused to agar media inoculated with MS, this phenomenon is similar to ability of CHX adsorbed and released from retention site (Fig. 4).

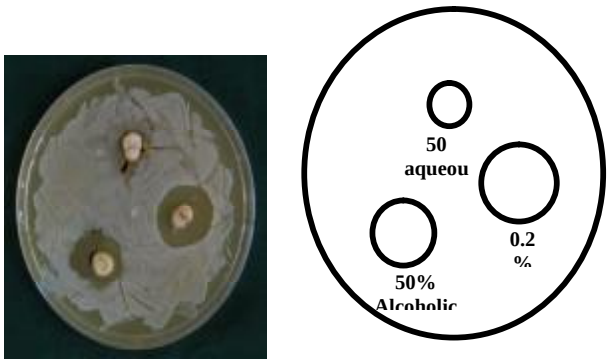

Figure 4: Ability of CHX adsorption on the crown surfaces of the tooth in comparison to the adsorption of Sage Alcoholic and Aqueous extract.

From the results shown above, it is quite obvious that the aqueous and alcoholic extracts of sage leaves had exerted antimicrobial action against Mutans streptococci but the aqueous extract was less effective than the alcoholic extract regarding the antibacterial effect against mutans streptococci while the minimum bactericidal concentration (MBC) of the aqueous extract was 50% and alcoholic extract was 20%. Reduction in viable count of MS requires higher concentration of aqueous extract in comparison to concentration alcoholic extract. Alcoholic and aqueous extracts interfered with some metabolic activities of MS including adherence on the smooth surface and acid production; the alcoholic extract of sage have the ability to adsorbed on the tooth surface and release from the retention sides (substantively).

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