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**ORIGINAL ARTICLE**

# Comparison of Antibacterial Properties of Salvia Officinalis with Commercially Available Mouth-Rinse

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## ABSTRACT

Dental caries, commonly known as cavities/tooth decay, is a widespread and one of the most prevalent diseases affecting people of all ages. Despite its prevalence, dental caries is largely preventable through good oral hygiene practices and reducing sugar intake. A variety of commercially available mouthwashes are being recommended by dental health professionals because of their antibacterial properties but their side effects limits the use of these mouth washes hence, plant based natural compounds are being evaluated for their antibacterial properties in the current study Salvia Officinalis was evaluated in this regard.

**Methodology:** It was an in-vivo preclinical experimental study conducted from October 2022 to January 2023. The calculated sample size was n =60. The participants were given an envelope for group randomization. The organisms were grown on appropriate media and extract was prepared and diluted. The extract was diluted in distilled water at 1:4 (Extract: Distilled water) concentration. Study participants were instructed to not brush their teeth before sampling. Study participants were divided into three groups (negative control, positive control, Salvia Officinalis extract rinse group) each group had 20 participants. Diluted SO extract was given to the experimental group for rinses, distilled water was given to the negative control group and positive controls were given a standard commercially available mouth rinse. The next sample of plaque was collected after two hours to observe the effects of SO extract on bacterial colonies.

**Results:** The pre and post-samples showed a significant (p-value <0.05) decrease in number of colonies in the positive control group (conventional rinse) and Salvia officinalis extract group. The intra-group comparison of negative and positive control showed a significant difference in number of colonies and the same was observed with the Salvia officinalis rinse. However, the positive control and Salvia officinalis extract comparison was insignificant.

**Conclusion:** The invitro experiment highlighted that the S. officinalis plant extract has equivalent effects against S. mutans and P. gingivalis as that of commercially available mouth rinse.

**Keywords:** Salvia officinalis**,** S. mutans, P. gingivalis, Moth rinse

## INTRODUCTION

Dental caries, commonly known as cavities/tooth decay, is a widespread and one of the most prevalent diseases affecting people of all ages (1). Dental caries results from the destruction of the tooth enamel, which is the hard, outer layer of the tooth, by the acid produced by various oral pathogens (2). The primary cause of dental caries is the accumulation of plaque, a sticky film of bacteria, over the teeth (3). When sugar and other carbohydrates in the diet interact with plaque, acid is produced, which can erode the enamel and cause dental caries (4). If untreated, dental caries can lead to periodontitis, inflammation of periodontal tissues, which is associated with pain, sensitivity, infection, and eventually, tooth loss (5). This makes it a significant public health concern, as it can impact oral health and overall well-being (6). Despite its prevalence, dental caries is largely preventable through good oral hygiene practices and reducing sugar intake (7). Regardless of the availability of effective treatments and preventive measures, dental caries remains a major public health concern, particularly in developing countries like Pakistan (8).

Dental caries is primarily caused by Streptococcus mutans and Lactobacillus, which are acid-producing bacteria that thrive in a sugary environment (9). These bacteria convert sugars from the diet into lactic acid, which erodes the enamel of the teeth and leads to the formation of cavities (10). Periodontitis, on the other hand, is caused by a group of bacteria known as the red complex, which includes Porphyromonas gingivalis, A. actinomycetemcomitans, Treponema denticola, and Tannerella forsythia (11). These bacteria collectively cause inflammation and damage to the gums and supporting structures of the teeth, leading to periodontitis (12). Hence, it's important to maintain good oral hygiene to prevent these conditions. Preventive measures may include brushing and flossing regularly and using antibacterial mouthwashes (13). A variety of commercially available mouthwashes are being recommended by dental health professionals because of their antibacterial properties and to improve conditions like halitosis (14). Chlorhexidine compounds and their various formulations are the primary antibacterial components of most of the commercially available mouth rinses (15). Apart from its antimicrobial effects, chlorhexidine-containing mouthrinses have multiple reported adverse effects (16). Associated adverse effects of chlorhexidine mouthwashes are brown or yellow discoloration of teeth, metallic taste after rinsing, dry mouth, irritation of oral mucosa, andrarey allergic reactions are also reported (17, 18).

Phytotherapy is a centuries-old practice of various global cultures to treat multiple ailments (19). Extracts of different parts of plants, including leaves, flowers, stems, roots and seeds, have active compounds with a diverse range of medicinal properties (20). Minimum adverse effects, cost-effectiveness, and significant medicinal properties of plant compounds are the prime reasons for the revival of phytomedicine (21). One of the perennial herbs Salvia officinalis, commonly known as sage is traditionally used as a remedy for various ailments (22). Due to its fragrant leaves, it is also used in various culinary dishes, including stuffings, salads, marinades, sauces, and teas (23). It is also used in aromatherapy and has been used for centuries to treat conditions such as indigestion, sore throat, and hot flashes associated with menopause (24). Additionally, sage is believed to have antimicrobial and anti-inflammatory properties, and some studies have shown that it may help manage certain cognitive and memory-related conditions (25).

In our study, we have compared the antibacterial properties of Salvia officinalis with commercially available mouthwash.

## METHODOLOGY

It was an in-vivo preclinical experimental study conducted at Baqai Medical and Dental College Karachi., from October 2022 to January 2023. The total calculated sample size was n =60.

Consecutive sampling technique was used to recruit the participants. The participants were given an envelope for group randomization. Film of plaque from the labial surface of teeth of study participants was collected on sterile strips that were transported to the laboratory for culture in sterile containers. For culture, S. mutant samples were inoculated in Columbia Agar with 5% sheep blood and incubated for 48 h at 37 °C and increased level of $CO_2$. P. gingivalis were grown in Wilkins-Chalgren anaerobic broth under anaerobic conditions of 5% CO2, 10% H2, and 85% N2 at 37 °C. All bacteria were subcultured twice and were grown to the early stationary phase. Leaves of Salvia officinalis (1000-gram) were purchased from the local market of Karachi and authentication number i.e. Specimen voucher 9102 was allotted. The leaves were washed and shed dried and lastly grinded to powder form. The leaves were soaked in 2500mL of 70% ethanol for 15 days with intermittent shaking. After 15 days the filtrate was filtered with Whatman filter paper (number 1) that was further processed at 60ºC by using a water bath. The mixture was then dried at 50ºC until a well-concentrated extract was produced on a rotary evaporator. The extract was kept in an airtight bottle and stored in a refrigerator till usage. The extract was diluted in distilled water at 1:4 (Extract: Distilled water) concentration. Study participants were instructed to not brush their teeth before submission of the plaque sample. Study participants were divided into three groups (negative control, positive control, and Salvia officinalis extract rinse group) each group had 20 participants. Diluted Salvia officinalis extract was given to the experimental group for rinses, distilled water was given to the negative control group and positive controls were given a standard commercially available mouth rinse. Next sample of plaque was collected after two hours to observe the effects of Salvia officinalis extract on bacterial colonies. ANOVA followed by post hoc Tukey's test was applied to identify the inter and intra-group comparison and Paired t-test was applied as a test of significance for pre and post experimental comparison, <0.05 p-value was considered as significant at 95% confidence interval.

## RESULTS

There were sixty participants in study 39 (65%) were males and 21 (35%) were females the mean age of participants was 28 ± 3.5. On asked about brushing habits 49 (81%) participants responded that they brush their teeth daily. Figure 1 depicts the demographic data of study participants. The growth of colonies was calculated on growth media plates in samples collected prior to rinsing and samples that were taken after the rinsing. The pre and post-samples showed a significant (p-value <0.05) decrease in number of colonies in the positive control group (conventional rinse) and Salvia officinalis extract group as shown in table 1. The intra-group comparison of negative and positive control showed a significant difference in number of colonies and the same was observed with the Salvia officinalis rinse. However, the positive control and Salvia officinalis extract comparison was insignificant. Table 2 shows the intra-group comparison of experiment.

Table 1: Paired t-test analysis showing the number of colonies before and after intervention

| | Negative Control | Positive control | Salvia officinalis extract |
|---|---|---|---|
| Streptococcus Mutans | | | |
| Before | $10 \pm 1 \times 10^4$ | $9 \pm 2 \times 10^4$ | $11 \pm 2 \times 10^4$ |
| After | $10 \pm 2 \times 10^4$ | $7 \pm 1 \times 10^4$ | $7 \pm 1 \times 10^4$ |
| P value | 1.000 | 0.001* | 0.041* |
| P. gingivalis | | | |
| Before | $15 \pm 2 \times 10^3$ | $14 \pm 2 \times 10^3$ | $18 \pm 3 \times 10^3$ |
| After | $16 \pm 2 \times 10^3$ | $8 \pm 2 \times 10^3$ | $11 \pm 2 \times 10^3$ |
| P value | 1.000 | 0.001* | 0.001* |
| *significant p-value | | | |

Table 2: ANOVA followed by post hoc tukeys`s Analysis on post-interventional results

| Groups wise comparison Streptococcus Mutans | | | | p- value |
|---|---|---|---|---|
| Negative control | $8.5 \times 10^4$ | Positive control | $7 \times 10^4$ | 0.002* |
| Negative control | $10 \times 10^4$ | Salvia officinalis extract | $6.7 \times 10^4$ | 0.003* |
| Positive control | $7 \times 10^4$ | Salvia officinalis extract | $6.7 \times 10^4$ | 0.125 |
| Groups wise comparison P. gingivalis | | | | |
| Negative control | $15 \pm 2 \times 10^3$ | Positive control | $9 \pm 2 \times 10^3$ | 0.001* |
| Negative control | $15 \pm 2 \times 10^3$ | Salvia officinalis extract | $10 \pm 2 \times 10^3$ | 0.023* |
| Positive control | $9 \pm 2 \times 10^3$ | Salvia officinalis extract | $10 \pm 2 \times 10^3$ | 0.390 |

*significant p-value

## DISCUSSION

The human oral cavity harbours multiple pathogens that initiate and progress various oral diseases including dental caries, gingivitis, and periodontitis. Different standard techniques like daily-brushing, flossing and rinsing with an antibacterial mouthwash are recommended by dental health care professionals to prevent oral diseases (26). In this study, we have compared the antibacterial effects of standard mouth wash containing chlorhexidine with the diluted extract of Salvia officinalis. The antibacterial properties were evaluated against two major microbes of the oral cavity, S. mutans and P. gingivalis which primarily reside in the biofilms that form on the tooth surface also known as dental plaque (27).

The pre and post-treatment results in the negative control group, treated with distilled water only, were non-significant showing no effect of distilled water on bacterial colonies. While in the standard group, treated with chlorhexidine containing mouth rinse, a significant decrease in bacterial colonies was observed that confirms antibacterial effects of chlorhexidine. While our experimental extract of Salvia officinalis showed maximum inhibition of both the colonies of S. mutans and P. gingivalis and showed better efficacy when compared with the standard mouthwash group. An in-vitro study conducted in South Africa reported that the essential oil of Salvia officinalis produced its antibacterial effect against S. mutans by penetrating bacterial cell walls and causing disruption (28). Another in-vitro study reported that methanolic extracts of different herbs including Salvia officinalis have antimicrobial properties, even at low concentrations, against S. mutans (29). Similar results were observed from another study that reported the pure sage oil had maximum inhibitory effects when compared with its methanolic extract and standard chlorhexidine mouthwash against various bacteria including Streptococcus mutans, Streptogas, Streptofeacalis, Staphylococcus aureus, Escherichia coli, Klebsiella sp., and Pseudomonas sp.) (30). Regarding P. gingivalis, another study reported similar results that showed promising antimicrobial activity of the S. officinalis dichloromethane crude extract against P. gingivalis (31). To the best of our knowledge, there is a lack of sufficient literature about the antibacterial activity of S. officinalis against P. gingivalis. But the available literature strongly supports our result findings that is exhibiting better antibacterial activity against S. mutans and P. gingivalis when compared with the chlorhexidine mouthwash.

The intergroup comparison in our results showed a significant difference between the negative control group (distill water treated) and positive control group (chlorhexidine mouthwash treated) showing notable antibacterial effects of chlorhexidine against both S. mutans and P. gingivalis. In another comparison, the Salvia officinalis group also exhibited a noteworthy inhibitory effect against S. mutans and P. gingivalis when compared with the group treated with distilled water only. A study that compared the chlorhexidine treated group with herbal treated group showed significant inhibition of bacteria in both groups by reducing plaque and improved gingivitis but the chlorhexidine group showed side effects like dryness of mouth and burning sensation. The herbal group did not show any such symptoms (32). Another study done in Italy compared a polyherbal mouthwash containing Salvia officinalis with a standard mouthwash as a placebo in patients with moderate to severe

periodontitis. After 3 months the Salvia officinalis-containing mouthwash was proved effective in reducing bleeding score and plaque accumulation when compared with placebo but no difference was reported on probing depth and clinical attachment level between the herbal mouthwash and chlorhexidine-containing mouthwash (33). Similar results were observed in another study reporting the inhibitory effects of Salvia officinalis essential oil against common oral pathogens (34). In our study, the chlorhexidine and Salvia officinalis groups showed similar antimicrobial activity when compared. Similar results were obtained from various previous studies showing equal efficacy of chlorhexidine mouthwash and essential oil of Salvia officinalis (35).

Literature has reported that S. officinalis has an aromatic taste and smell and it also does not produce chlorhexidine-like adverse effects including tooth discoloration, dry mouth, metallic taste, and irritation of oral mucosa (36, 37). Therefore, being less toxic and having equal antimicrobial effects as of commercially available chlorhexidine mouthwash, the herbal formulation of S. officinalis can be a better alternative or adjunctive to the standard mouthwashes. This may increase the antibacterial efficacy and will improve compliance because of lesser side effects.

## CONCLUSION

Taken together, the invitro experiment highlighted that the S. officinalis plant extract has equivalent effects against S. mutans and P. gingivalis. Further studies are recommended to validate its use as mouth rinse.

**Conflict of interest:** None

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