Comparison of effects of *Azadirachta indica, Salvia officinalis* and commercial mouth rinse on *Streptococcus Mutans* and *P. gingivalis*

Mehak Mussawar[1], Muhammad Tariq Fayyaz[2], Shumail Gul[1*], Juweria Abid[3], Aneeqa Jawaid[4], Abdul Momin Rizwan Ahmad[3]

[1]Department of Pathology, Baqai Medical University
[2]Department of Dental Materials, Karachi medical and dental college.
[3]Department of Nutrition and Dietetics, National University of Medical Sciences
[4]Department of Oral Biology, Baqai Medical and University.

***Corresponding author:**
Dr. Shumail Gul
BDS
Lecturer
Department of Pathology, Baqai Medical University
shumail_gul@yahoo.com

Abstract:

Background: Antiulcer, antimalarial, antifungal, antiviral, antimutagenic, anticarcinogenic, hypoglycemic and immunomodulatory properties of *Azadirachta indica* leaves extract have been validated in the literature along with that different components of the Neem tree have shown potent antibacterial activity against various bacteria types. Similarly various studies have documented different health benefits and antibacterial properties of *Salvia officinalis*. Therefore, in current study antibacterial activity of chlorhexidine-containing mouthwash with leaf extracts of natural herbs, *Azadirachta indica,* and *Salvia officinalis* were compared against *Streptococcus Mutans and P. gingivalis.*

Methodology: Invtro experimental study was conducted in the tertiary care hospital of karachi. The extract of plants were prepared by rotary evaporator. The extracts were 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 four groups (negative control, positive control, *A. indica,* and *S. officinalis* extract rinse group) each group had 20 participants. Diluted *A. indica* and *S. officinalis* extract rinses were 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 *A. indica* and *S. officinalis* extract rinses on bacterial colonies. Bacteria were cultured in their appropriate media in optimized habitat.

Results: The pre and post-samples showed a significant (p-value <0.05) decrease in the number of colonies in the positive control group (conventional rinse) *A. indica* and *S. officinalis* extract rinses group.

The intragroup comparison of negative and positive control showed a significant difference in the number of colonies and the same was observed with the neem leaf extract rinse. However, the positive control, *A. indica*, and *S. officinalis* extract rinses comparison was insignificant.

Conclusion: *A. indica*, and *S. officinalis* have promising antimicrobial activity, especially against oral pathogens including *S. mutans* and *P. gingivalis that are*

Keywords: *A. indica, S. officinalis,* antimicrobial activity, *S. mutans, P. gingivalis*

Introduction:

For many years, people have turned to herbal treatment for various ailments (1). From traditional Chinese medicine, and Greek medicine to Ayurveda in India, the use of various herbs have been a cornerstone of natural healing practices for multiple diseases around the world (2). In recent times, the popularity of herbal remedies has only continued to grow, with many people looking for alternative treatments to traditional standard medications (3). In our study, we have compared the antibacterial activities of *Azadirachta indica* and *Salvia officinalis*.

Azadirachta indica, commonly known as 'Neem-tree', is a very common plant in the dry regions of the world, especially in Pakistan, India, and China (4). This plant belongs to the Meliaceae family and its flowers, leaves, stem bark, and seeds have numerous medicinal properties (5). Neem tree is also known as the *divine tree* and *village pharmacy* in the south Asian region due to its various healthpromoting effects (6). Studies have reported antiulcer, antimalarial, antifungal, antiviral, antimutagenic, and anticarcinogenic, hypoglycemia and immunomodulatory properties of *Azadirachta indica* leaves extract (7-9). Therapeutic effects of Neem twigs have been reported against endothelial dysfunction, leprosy, diabetes, and system inflammation (10). Different components of the Neem tree have shown potent antibacterial activity against various bacteria types (11). *Salvia officinalis*, a perennial herb, is native to the Mediterranean region but is widely cultivated around the world (12). *Salvia officinalis*, commonly known as *sage*, has multiple therapeutic effects and has been used in traditional medicine for decades (13). Sage has been used to improve sore throat, perspiration, hot flashes in menopause, indigestion, dyslipidemia, and mental impairment (14). Recent studies have reported that the extracts from *Salvia officinalis* have bactericidal activity against *Staphylococcus aureus, Escherichia coli, Salmonella*

typhimurium, and various antibiotic-resistant strains (15). The antimicrobial activity of Sage is thought to be due to the presence of bioactive components such as rosmarinic acid and thujone in it (15).

S. mutans and *P. gingivalis* are commonly associated with the occurrence and progression of dental diseases such as dental caries, gingivitis, and periodontitis (16). *S. mutans* metabolizes sugars and produces acids that lead to the erosion of enamel and cause dental caries (17). *P. gingivalis* is strongly associated with periodontitis, chronic inflammation of the gums leading to tooth loss (18). Various antibacterial mouth rinses are available in the market that contains chlorhexidine. Chlorhexidine is a broad-spectrum antibacterial agent that is effective against a diverse range of microbes, including *S. mutans* and *P. gingivalis* (19). Chlorhexidine-containing mouthwashes are recommended by dental health professionals as an adjunct to conventional brushing and flossing but patient compliance is not up to the mark due to various adverse effects of standard mouthwashes (20). Chlorhexidine can cause brown staining over the teeth, restorations, and oral appliances such as braces or dentures (21). It may also develop a metallic or bitter taste and sometimes lead to dry mouth or a burning sensation over the oral mucosa (22). Apart from oral mucosal irritation, some people have also reported allergic reactions leading to rash, hives, and shortness of breath (23).

In our study, we have compared the antibacterial activity of chlorhexidine-containing mouthwash with leaf extracts of natural herbs, *Azadirachta indica,* and *Salvia officinalis,* which have minimal adverse effects and may improve patient compliance.

Methodology:

From December 2022 to February 2023, an in-vivo preclinical experimental investigation was carried out at Karachi's tertiary care hospital. N = 80 was the estimated sample size. The participants were chosen using a sequential sampling procedure. For group randomization, an envelope was provided to each participant. Film of plaque from the labial surface of the 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 the 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 *A. indica* and *S. officinalis* (1000-gram) were purchased from the local market of Karachi and an authentication number i.e. Specimen vouchers 1081 and 1082 were allotted respectively. The leaves were washed and shed dried and lastly ground 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 60^{o}C by using a water bath. The mixtures were then dried at 50^{o}C until a well-concentrated extract was produced on the rotary evaporator. The extracts were kept in an airtight bottle and stored in a refrigerator till usage. The extracts were 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 four groups (negative control, positive control, *A. indica,* and *S. officinalis* extract rinse group) each group had 20 participants. Diluted *A. indica* and *S. officinalis* extract rinses were 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 *A. indica* and *S. officinalis* extract rinses on bacterial colonies. ANOVA followed by post hoc Tukey's test was applied to identify the inter and intragroup 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 a 95% confidence interval.

Results:

There were 80 participants in the study 59 (73%) were males and 21 (27%) were females the mean age of participants was 28 ± 2.31. On asking about brushing habits 51 (63.7%) participants responded that they brush their teeth once a day. The growth of colonies was calculated on growth media plates in samples collected before rinsing and samples that were taken after the rinsing. The pre and post-samples showed a significant (p-value <0.05) decrease in the number of colonies in the positive control group (conventional rinse) *A. indica* and *S. officinalis* extract rinses group as shown in table 1. The intragroup comparison of negative and positive control showed a significant difference in the number of colonies and the same was observed with the neem leaf extract rinse. However, the positive control, *A. indica,* and *S. officinalis* extract rinses comparison was insignificant. Table 2 shows the intragroup comparison of the experiment.

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

	Negative Control	**Positive control**	***A. indica* extract**	***S. officinalis***
Streptococcus Mutans				
Before	11 ± 1.4 x 10^4	9 ± 2.1 x 10^4	12 ± 24 x 10^4	11.31 x 10^4

After	$10 \pm 2 \times 10^4$	$4 \pm 2.1 \times 10^4$	$5 \pm 3.1 \times 10^4$	5.61×10^4
P value	0.518	0.001*	0.001*	0.001*
P. gingivalis				
Before	$19 \pm 1.8 \times 10^3$	$18 \pm 3.1 \times 10^3$	$18 \pm 2.9 \times 10^3$	$17 \pm 4.2 \times 10^3$
After	$19 \pm 2.1 \times 10^3$	$12 \pm 1.2 \times 10^3$	$10 \pm 3.6 \times 10^3$	$11 \pm 2.3 \times 10^3$
P value	0.538	0.001*	0.001*	0.001*
*significant p-value				

Table 2. ANOVA followed by post hoc Tukey's Analysis on post-interventional results

Groups wise comparison *Streptococcus Mutans*				**p-value**
Negative control	$10 \pm 2 \times 10^4$	**Positive control**	$4 \pm 2.1 \times 10^4$	0.001*
Negative control	$10 \pm 2 \times 10^4$	***A. indica* extract**	$5 \pm 3.1 \times 10^4$	0.001*
Positive control	$4 \pm 2.1 \times 10^4$	***A. indica* extract**	$5 \pm 3.1 \times 10^4$	0.341
Negative control	$10 \pm 2 \times 10^4$	***S. officinalis***	5.61×10^4	0.001*
Positive control	$4 \pm 2.1 \times 10^4$	***S. officinalis***	5.61×10^4	0.517
A. **indica extract**	$5 \pm 3.1 \times 10^4$	***S. officinalis***	5.61×10^4	1.000
Groups wise comparison *P. gingivalis*				
Negative control	$19 \pm 2.1 \times 10^3$	**Positive control**	$12 \pm 1.2 \times 10^3$	0.005*
Negative control	$19 \pm 2.1 \times 10^3$	***A. indica* extract**	$10 \pm 3.6 \times 10^3$	0.001*
Positive control	$12 \pm 1.2 \times 10^3$	***A. indica* extract**	$10 \pm 3.6 \times 10^3$	0.815
Negative control	$19 \pm 2.1 \times 10^3$	***S. officinalis***	$11 \pm 2.3 \times 10^3$	0.001*
Positive control	$12 \pm 1.2 \times 10^3$	***S. officinalis***	$11 \pm 2.3 \times 10^3$	0.927
A. **indica extract**	$10 \pm 3.6 \times 10^3$	***S. officinalis***	$11 \pm 2.3 \times 10^3$	0.918

*significant p-value

Discussion:

The oral cavity harbors various bacteria that initiate and progress oral diseases such as dental caries, gingivitis, and periodontitis. *Azadirachta indica* and *Salvia officinalis* have been reported vastly for their medicinal activities against various diseases, especially their antibacterial properties. Our results showed that both the herbal extracts significantly declined the *S. mutans* and *P. gingivalis* colonies when compared with locally available chlorhexidine-containing mouthwash. *S. mutans* is a major contributor to tooth decay as being a potent initiator of dental caries (24). The second bacteria of our study, *P. gingivalis*, also has a vital role in the pathogenesis of periodontitis which is an inflammatory disease of supporting dental tissues (25). Upon comparing the number of bacterial colonies of both *S. mutans* and *P. gingivalis,* the group treated with only distilled water showed no significant difference as expected. People treated with chlorhexidine-containing mouthwash showed a significant decline in the number of both *S. mutans* and *P. gingivalis* colonies which expresses the notable antibacterial activity of chlorhexidine. Various other studies have also reported the antibacterial activity of chlorhexidine against *S. mutans* and *P. gingivalis* (26-28). The group treated with a diluted extract of *A. indica* showed more potent and remarkable antibacterial activity against both *S. mutans* and *P. gingivalis* when compared with the chlorhexidine group. A study has also reported that the Neem leaves extract has shown significant zones of inhibition against *S. mutans* when compared with the conventional chlorhexidine mouthwash (29). Another study has reported that *A. indica* has considerable antibacterial activity against *S. mutans* (30). Similar results were observed when *A. indica,* at different doses, was experimented against *S. mutans (31).* Regarding inhibition of *P. gingivalis,* multiple previous studies have reported comparable results showing significant inhibition when treated with *A. indica*. One of those studies reported that the neem extract has a potent antimicrobial effect against *P. gingivalis* (32). Another invitro study has concluded that *A. indica* oil has bacteriostatic activity against *A. actinomycetemcomitans*, *P. gingivalis* and *F. nucleatum* and Neem oil can be a better alternative for the management of alveolitis (33). Similar results were observed from another study that reported that *A. indica* caused a significant reduction in bleeding on probing, pocket depth, plaque accumulation, and total leucocyte count along with interleukin-6 in gingival crevicular fluid in periodontitis-induced rabbit model (34).

Like *A. indica, Salvia officinalis* also showed a notable reduction in bacterial colonies of both *S. mutans* and *P. gingivalis. S. officinalis* showed better antimicrobial activity when compared with the standard

chlorhexidine. Multiple other studies have also reported similar results showing the inhibitory effects of *S. officinalis* against various bacteria. A study has reported that *S. officinalis* essential oil succeeded to penetrate the bacterial cell wall of *S. mutans* and causing cell disruption (35). Another study documented the direct inhibitory effects of *S. officinalis*-containing GIC against *S. mutans* and *L. casei* in a dose-dependent manner (36). Mirroring results were stated in another study showing the antibacterial activity of the methanolic extract of *S. officinalis* against *S. mutans* even at very low concentrations (37). The antimicrobial activity of *S. officinalis* was stated in another study that showed significant inhibition of various periodontopathogens including *P. gingivalis*, *A. actinomycetemcomitans*, *P. nigrescens*, *F. nucleatum*, *P. intermedia* and *T. denticola* (38). A clinical trial done at the university of Baghdad showed the potential anti-inflammatory and antibacterial activity of *S. officinalis* gel in patients with chronic periodontitis (39). An equivalent antibacterial activity was observed against both *S. mutans* and *P. gingivalis* by *S. officinalis* when compared with standard chlorhexidine-containing mouthwash (40).

The intragroup comparisons in our study also reported similar results showing a significant difference between the negative control group treated only with distilled water and all other groups including the chlorhexidine group, *A. indica* group, and *S. officinalis* group. The intragroup comparison between the chlorhexidine group, *A. indica* group, and *S. officinalis* group showed no significant difference that states that our herbs have a better or equivalent antimicrobial activity as compared to the standard chlorhexidine.

Conclusion:

Following our results and previous literature we can conclude that both of our herbs, *A. indica*, and *S. officinalis* have promising antimicrobial activity, especially against oral pathogens including *S. mutans* and *P. gingivalis.* Formulations of these herbal leaves can be used in mouthwashes for their antimicrobial activity. These herbal formulations can be a better alternative to standard chlorhexidinecontaining mouth rinse that have very low compliance due to multiple adverse effects.

References

1. Nugraha RV, Ridwansyah H, Ghozali M, Khairani AF, Atik N. Traditional herbal medicine candidates as complementary treatments for COVID-19: a review of their mechanisms, pros and cons.
Evidence-Based Complementary and Alternative Medicine. 2020;2020.
2. Buell PD, Anderson EN. Other Medicines, Mainstream and Not, and the HHYF. Arabic Medicine in China: Brill; 2021. p. 54-8.
3. Shedoeva A, Leavesley D, Upton Z, Fan C. Wound healing and the use of medicinal plants. Evidence-Based Complementary and Alternative Medicine. 2019;2019.
4. Moin MS, Siddiqui JI, Alam MA, Khatoon F, Khan S, Minhajuddin A. Ethnomedicinal potential of widely used plant Azadirachta indica A. Juss: A comprehensive review. The Journal of Phytopharmacology. 2021;10(6):456-67.
5. Mbembo BMW, Ashande CM, Shotsha LA, Ngunde SNT, Masasi BM, Dipa JT, et al. A Minireview on the Phytochemistry and Pharmacobiology of Azadirachta indica A. Juss.(Meliaceae):
Towards future research directions. Journal of Complementary and Alternative Medical Research. 2021;15(2):1-21.
6. Islas JF, Acosta E, Zuca G, Delgado-Gallegos JL, Moreno-Treviño MG, Escalante B, et al. An overview of Neem (Azadirachta indica) and its potential impact on health. Journal of Functional Foods.
2020;74:104171.
7. Asghar HA, Abbas SQ, Arshad MK, Jabin A, Usman B, Aslam M, et al. Therapeutic Potential of Azadirachta indica (Neem)-A Comprehensive Review. Sch Int J Tradit Complement Med. 2022;5:47-64.
8. Hasan A. AZADIRACHTA INDICA AND ITS MEDICINAL USE.
9. Chakrabortty P. A study on comparison of chemical composition of Azadirachta indica (neem) and Moringa oleifera (Sojina). Chattogram Veterinary and Animal Sciences University Chattogram4225, Bangladesh; 2021.
10. Bhamare UU, Mali YS, Shaikh AZ. Neem: As a natural medicine. Research Journal of Pharmacognosy and Phytochemistry. 2020;12(4):245-55.
11. Altayb HN, Yassin NF, Hosawi S, Kazmi I. In-vitro and in-silico antibacterial activity of Azadirachta indica (Neem), methanolic extract, and identification of Beta. d-Mannofuranoside as a promising antibacterial agent. BMC Plant Biology. 2022;22(1):1-14.
12. Tursun AO, Sipahioglu HM, Telci I. Genetic relationships and diversity within cultivated accessions of Salvia officinalis L. in Turkey. Plant Biotechnology Reports. 2021;15(5):663-72.
13. Al-Mijalli SH, Assaggaf H, Qasem A, El-Shemi AG, Abdallah EM, Mrabti HN, et al. Antioxidant, Antidiabetic, and Antibacterial Potentials and Chemical Composition of Salvia officinalis and Mentha suaveolens Grown Wild in Morocco. Advances in Pharmacological and Pharmaceutical Sciences. 2022;2022.
14. Jakovljević M, Jokić S, Molnar M, Jašić M, Babić J, Jukić H, et al. Bioactive profile of various Salvia officinalis L. preparations. Plants. 2019;8(3):55.
15. Mokhtari R, Kazemi Fard M, Rezaei M, Moftakharzadeh SA, Mohseni A. Antioxidant, Antimicrobial Activities, and Characterization of Phenolic Compounds of Thyme (Thymus vulgaris L.), Sage (Salvia officinalis L.), and Thyme–Sage Mixture Extracts. Journal of Food Quality. 2023;2023.

16. Park S-H, Kim K, Cho S, Chung D-H, Ahn S-J. Variation in adhesion of Streptococcus mutans and Porphyromonas gingivalis in saliva-derived biofilms on raw materials of orthodontic brackets.
Korean Journal of Orthodontics. 2022;52(4):278-86.
17. Alejandra BM, Daniel OM. Virulence factors of Streptococcus mutans related to dental caries. Staphylococcus and Streptococcus. 2020;11:9.
18. Xu W, Zhou W, Wang H, Liang S. Roles of Porphyromonas gingivalis and its virulence factors in periodontitis. Advances in protein chemistry and structural biology. 2020;120:45-84.
19. Kutsevol N, Virych P, Nadtoka O, Virych P, Krysa V. Synthesis of polymeric hydrogels incorporating chlorhexidine as potential antibacterial wound dressings. Molecular Crystals and Liquid Crystals. 2021;720(1):65-71.
20. Sharma K, Acharya S, Verma E, Singhal D, Singla N. Efficacy of chlorhexidine, hydrogen peroxide and tulsi extract mouthwash in reducing halitosis using spectrophotometric analysis: A randomized controlled trial. Journal of clinical and experimental dentistry. 2019;11(5):e457.
21. Soheilifar S, Khodadadi H, Naghdi N, Farhadian M. Does a diluted chlorhexidine-based orthodontic mouthwash cause less discoloration compared to chlorhexidine mouthwash in fixed orthodontic patients? A randomized controlled trial. International Orthodontics. 2021;19(3):406-14. 22. Nezam S, Singh P, Ojha R, Khan SA, Kumari N, Kumari N. Evaluation of the Antimicrobial Activity of Magnetized Water and Its Comparison with Chlorhexidine 0.2% in Young Children for 3 Weeks. The Journal of Contemporary Dental Practice. 2022;23(1):83-8.
23. Gerges BZ, Rosenblatt J, Truong Y-L, Reitzel RA, Raad I. Review of allergic reactions from use of chlorhexidine on medical products in clinical settings over 40 years: Risks and mitigations.
Infection Control & Hospital Epidemiology. 2022;43(6):775-89.
24. Lin Y, Chen J, Zhou X, Li YJCrim. Inhibition of Streptococcus mutans biofilm formation by strategies targeting the metabolism of exopolysaccharides. 2021;47(5):667-77.
25. Bunte K, Kuhn C, Walther C, Peters U, Aarabi G, Smeets R, et al. Clinical significance of ragA, ragB, and PG0982 genes in Porphyromonas gingivalis isolates from periodontitis patients.
2021;129(3):e12776.
26. Nomura R, Inaba H, Matayoshi S, Yoshida S, Matsumi Y, Matsumoto-Nakano M, et al. Inhibitory effect of a mouth rinse formulated with chlorhexidine gluconate, ethanol, and green tea extract against major oral bacterial species. Journal of Oral Science. 2020;62(2):206-11.
27. O'Connor M, Harrison G, Lenahan D, Moran GP. A dentifrice containing salivary enzymes and xylitol exhibits superior antimicrobial activity in vitro against adherent Streptococcus mutans compared to a chlorhexidine dentifrice. Letters in Applied Microbiology. 2023;76(2):ovad026. 28.
Khobragade VR, Vishwakarma PY, Dodamani AS, Jain VM, Mali GV, Kshirsagar MM. Comparative evaluation of indigenous herbal mouthwash with 0.2% chlorhexidine gluconate mouthwash in prevention of plaque and gingivitis: A clinico-microbiological study. Journal of Indian Association of Public Health Dentistry. 2020;18(2):111.
29.Christy S, Nivedhitha M. Antimicrobial Efficacy of Azadirachta indica against Streptococcus mutans-An In vitro Study. Asian Journal of Pharmacy and Technology. 2019;9(3):149-53.

30.Kumari PD, Shenoy SM, Khijmatgar S, Chowdhury A, Lynch E, Chowdhury CR. Antibacterial activity of new atraumatic restorative treatment materials incorporated with Azadirachta indica (Neem) against Streptococcus mutans. Journal of oral biology and craniofacial research. 2019;9(4):321-5. 31.

Shah PH, Mamtha M, Bhatt V, Zatakiya D, Shah SH, Gandhi B. Effect Of Antimicrobial Activity Of Herbal Medicines On Streptococcus Mutans. National Journal of Integrated Research in Medicine. 2019;10(6).

32. Müller-Heupt LK, Vierengel N, Groß J, Opatz T, Deschner J, von Loewenich FD. Antimicrobial activity of Eucalyptus globulus, Azadirachta indica, Glycyrrhiza glabra, Rheum palmatum extracts and rhein against Porphyromonas gingivalis. Antibiotics. 2022;11(2):186.
33. Marie Elvire N, Hortense G, Emmanuel N, Rose N, Francine M. Evaluation of the in vitro antibacterial activity of Azadirachta indica used for the treat-ment of alveolitis. Int J Biopharm Sci. 2020;2(2):119.
34. Shahzad M, Ali L, Johnson N. Effectiveness of Azadirachta indica bark extract in periodontitis induced rabbit model. Journal of Pharmacognosy and Phytochemistry. 2020;9(2):15-21.
35. Ntondini SS, Lenetha G, Dzogbewu TC. Antimicrobial activity of Salvia officinalis against Streptococcus mutans causing dental implant failure: An in vitro study. Journal of International Oral Health. 2021;13(5):499.
36. Shahriari S, Barekatain M, Shahtalebi MA, Farhad SZ. Evaluation of preventive antibacterial properties of a glass-ionomer cement containing purified powder of Salvia officinalis: An In vitro Study. International journal of preventive medicine. 2019;10.
37. Gylan EMA, Muharram BA, Ismail A, Al-Kholani M, AL-Haddad KA, Al-Akwa AAY, et al. IN VITRO EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF FIVE HERBAL EXTRACTS AGAINST STREPTOCOCCUS MUTANS. Universal Journal of Pharmaceutical Research. 2022;7(1):37-44.
38. Mendes FSF, Garcia LM, da Silva Moraes T, Casemiro LA, de Alcantara CB, Ambrósio SR, et al. Antibacterial activity of salvia officinalis L. against periodontopathogens: An in vitro study. Anaerobe. 2020;63:102194.
39. Ismael IW. Potential Anti-inflammatory Effect of Salvia Officinalis Gel as Adjunct to Scaling and Root Planing in Patients with Chronic Periodontitis (A Clinical Trial): University of Baghdad; 2020.
40. Haider AK, Naseem SB, Iqbal Z, Ashar F, Kaif MZ, Fayyaz MT. Comparison of Antibacterial Properties of Salvia Officinalis with Commercially Available Mouth-Rinse. Pakistan Journal of Medical & Health Sciences. 2023;17(01):372-.