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Original article

In vitro Antimicrobial Activity of *Salvadora persica* (Miswak) and *Syzigium aromaticum* (Clove) Extracts against Dental Plaque Pathogens

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Abstract:

- ***Background*:** Some periodontal bacteria on tooth plaque were shown to be sensitive to extracts from *Syzigium aromaticum* and *Salvadora persica*. The study aimed to assess the antimicrobial effect of *Syzigium aromaticum* and *Salvadora persica* extracts against dental plaque pathogens.
- ***Methods:*** Oral swabs were collected from 35 patients with dental plaque, and isolated microorganisms were identified using standard bacteriological methods. *Syzigium aromaticum* and *Salvadora persica* extracts were tested against dental plaque microorganisms on Muller Hinton agar at 50% concentration using the paper disks-diffusion technique to measure antimicrobial activity and inhibition zones.
- ***Result:*** From 35 dental plaque samples, 46 microorganisms were isolated, including *Staphylococcus aureus* (18), *Staphylococcus epidermidis* (3), *Streptococcus mutans* (6), *Streptococcus viridans* (12), and single isolates of *Pseudomonas aeruginosa*, *Proteus spp*., *Bacillus spp*. Two isolates each of *Actinobacillus actinomycetemcomitans* and *Candida albicans* were found. The study revealed that *Syzigium aromaticum* and *Salvadora persica* extracts possess antibacterial and antifungal properties against both gram-positive and gram-negative bacteria, as well as fungi.
- ***Conclusions:*** *Syzigium aromaticum* and *Salvadora persica* extracts demonstrated strong antimicrobial efficacy, suggesting their potential for oral hygiene treatment and prevention.
- **Keywords:** Antimicrobial activity, dental plaque, *Candida albicans*, *Syzigium aromaticum*, *Salvadora persica*

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1. INTRODUCTION

Biofilm formation (dental plaque) of microorganisms on the surface of the tooth, is a significant contributor to the onset and development of the two most common oral disorders, dental caries, and periodontal disease [1]. Control of plaque accumulation by using natural antimicrobial mouth rinses are essential for optimal oral health and is an important part of overall health and oral hygiene., mouth and tooth cleaning prevents many health problems [2, 3].

It has been documented that there are several plants utilized to treat dental diseases. These plants provide significant antioxidants, anti-inflammatory, and antibacterial action against various microorganisms, and in comparison, to standard treatments, they have fewer side effects [4].

Salvadora persica, often known as Miswak in Islamic cultures, belongs to the *Salvadoraceae* family and is used as a chewing stick or toothbrush tree [5]. Miswak for oral hygiene was advised as a Sunnah by the Prophet Mohammad (peace be upon him) [2]. Significant antibacterial and antifungal activity of *Salvadora persica* has been reported [6]. Miswak has been utilized in numerous forms, such as sticks, extracts, and toothpaste, for its powerful effects, and is widely used owing to its availability and inexpensive cost, or as a traditional practice. The presence of benzyl isothiocyanate in this plant is thought to be a key factor in preventing acid formation and bacterial development. It also has antiviral and antifungal properties [3].

Cloves, also known as *Syzygium aromaticum*, are dried aromatic unopened flower buds from the *Myrtaceae* family, used as spice all over the world and their oil has two major components, eugenol and β-caryophyllene. The eugenol has bactericidal effects which disrupt the cytoplasmic membrane of bacterial cells, so extracts from *Syzygium aromaticum* are effective against the majority of gram-positive and gram-negative bacteria [7]. Clove oil is used to relieve pain and found that clove and clove bud oil show potential antibacterial action against microorganisms that cause dental caries [8]. The aim of our study focuses on the antimicrobial activity of *Salvadora persica* and *Syzigium aromaticum* against oral plaque germs.

2. PATIENT and METHOD

This study was conducted in Research Center, Erbil Polytechnic University, and was approved by Ethics Committee.

2.1 Materials

In Erbil City, Kurdistan, Iraq, chewing sticks of *Salvadora persica* and *Syzygium aromaticum* were purchased in a local market. Thirty-five dental plaque oral swabs were collected from patients clinically diagnosed with chronic periodontitis were attended the periodontal clinics at Khanzad Health Center in Erbil City, Kurdistan, Iraq. All patients were questioned for age, occupation, and use of toothbrushes.

2.1.1 Culture Media

All samples were cultured in Blood agar (oxide); MacConkey agar (oxide), Trypticase soy agar plates supplemented with yeast extract, blood, vancomycin (TSV) and incubated aerobically and anaerobically at 37°C for 24-72hrs, Nutrient broth were used for bacterial suspension and Mueller- Hinton agar was used for sensitivity test. Colony morphology and API 20 E and API *Staph* were used to identify the isolated bacteria.

2.2 Methods

Thirty-five dental plaque oral swabs were cultured immediately on Tryptone Soya Vancomycin Agar with blood which enriched is media recommended for the selective isolation and identification of *Actinobacillus actinomycetemcomitans,* for 3 days, the plates were incubated anaerobically at 37°C. whereas for isolation of other bacteria, Blood agar and MacConkey agar were employed, and they were incubated aerobically for 24 hours at 37C°. Isolation and identification were done by using API 20E and API Staphylococcus. Identification of *Actinobacillus actinomycetemcomitans* done according to colony morphology on the media that are freshly isolated from patients has fimbriae and inner star-shaped.

2.2.1 Preparation of *Salvadora persica and Syzygium aromaticum* Extracts

Salvadora persica chewing sticks were broken into small pieces and ground into powder in a ball mill to make the aqueous extract. The dried buds of the flower *Syzygium aromaticum* were cleansed and rinsed with sterile distilled water before being shade dried and pulverized using a mechanical grinder. Both plant powders were weighed into a 10-gm quantity and mixed with 100 mL of sterile deionized distilled water in a sterile screw-capped container. After soaking for 72 hours at 4°C, the extracts were centrifuged for 15 minutes at 3000 rpm. The supernatant was filtered through a Whatman No. 1 filler paper, and extracts of both plants were prepared at a 50% concentration and stored in sterile screw-capped vials in the refrigerator until needed.

2.2.2 Preparation of Microorganisms

The density of three to four colonies of microorganisms suspended in nutrient broth was adjusted to 0.5 McFarland standards.

2.2.3 Antimicrobial Assay of *Salvadora persica and Syzygium aromaticum* extracts by using filter paper method

Filter paper discs with a diameter of 5 mm were prepared and sterilized using sterile forceps dipped in 95% ethanol. A 0.1ml inoculum of isolated microorganisms was inoculated to Muller-Hinton agar (containing approximately 10^5 bacteria). The discs were dipped in appropriate concentration and placed over plates. The plates were incubated at 37°C for 3 days

anaerobically and 24 hours aerobically. The diameter of inhibitory zones in millimeters was used to determine the activity of extracts. As a control, sterile deionized distilled water was used.

3. Results and Discussions

The gender and age distribution of patients were studied (Table 1). Of the total 35 patients, 48.6% were female and 51.4% were male. The age groups (50-59) and (60-69) years old had the highest prevalence of dental plaque, according to our findings males are more sensitive than females, these results agree with other studies that found that approximately 100% of adults and 60–90% of school children have dental decay, while 20%–50% of middle-aged individuals (35–44 years) have severe periodontal disease, which can lead to tooth loss. These findings suggested that a lack of understanding of oral hygiene guidelines, a crucial need for preventive, conservative, and prosthetic dental treatments, or risk factors in these age groups, such as poor hygiene and smoking, could all be contributing factors [9, 10].

Table 1. Age and gender distribution of patients

Age group	Gender				Total	
	Female		Male			
	No.	%	No	%	No.	%
19-29	2	11.8%	2	11.1%	4	11.2%
30-39	2	11.8%	3	16.7%	5	14.3%
40-49	5	29.4%	1	2.9%	6	17.1%
50-59	6	35.3%	3	16.7%	9	25.7%
60-69	2	11.8%	9	50%	11	31.4%
Total	17	48.6%	18	51.4%	35	100%

In the present study, the numbers of brushing teeth according to gender were studied. The results in (Table 2) showed that a larger percentage of females16 (72.7%) had a daily tooth brushing regimen as compared to males 5 (38.5%). According to another survey, 69 (36.3%) of females and 37(33.6%) of males used a toothbrush to brush their teeth [11]. Another study found that about 65% (36% females and 29% males) had daily oral hygiene [12]. Whereas, our result was obtained that 17.1% (23.1 males and 13.6% females) of subjects never cleaned their teeth. Only 36 (19%) of females and 30 (27.2%) of males have an awareness of oral health, according to 2016 research [11]. According to the Amish population in the United States, 2.6 % of the population has never cleaned their teeth. Oral hygiene habits of a given community were impacted by factors such as cultural background, religious norms, awareness of the issues that lack cleanliness creates, knowledge of the presence of certain cleaning instruments, education levels, and socioeconomic position [13]. People of lower socioeconomic status have fewer resources to address oral health issues: less free time, and money to buy toothpaste and

toothbrushes, resulting in poor oral hygiene due to a lack of proper dental knowledge, leading to an increase in the prevalence of dental caries around the world, with treatment being expensive [14, 15].

Table 2. Distribution of Tooth brushing/ day about gender

Tooth brushing/ day	Gender				Total	
	Female		Male			
	No.	%	No.	%	No.	%
Daily	16	72.7%	5	38.5%	21	60%
Week	2	9.1%	2	15.4%	4	11.4%
Rarely	1	4.6%	3	23.1%	4	11.4%
Never	3	13.6%	3	23.1%	6	17.1%
Total	19	62.9%	16	37.14%	35	100%

A total of 46 microorganisms were isolated from 35 dental plaque samples of which 18 isolates were *Staphylococcus aureus*, 3 isolates were *Staphylococcus epidermidis*, 6 isolates were *Streptococcus mutans*, 12 isolates were *Streptococcus viridans*, 1 isolate each of *Pseudomonas spp*., *Proteus spp., Bacillus spp*., 2 isolates were *Actinobacillus actinomycetemcomitans* and 2 isolates were *Candida albicans.* These findings were in line with previous research, which found that *Staphylococcus aureus* was the most common microbe isolated from study subjects' oral swabs, followed by *Streptococcus mutans* and *Candida albicans* [15-17]. The remaining food debris and saliva combine with oral microbes to produce a thick white film on the tooth known as dental plaque, which releases acid, causing tooth surface damage and the formation of holes and cavities [15].

Table 3. The antimicrobial activities of *Salvadora persica* and *Syzygium aromaticum* extract on isolated microorganisms.

Microorganisms	No.	*Salvadora persica*	*Syzygium aromaticum*
		Zone of inhibition/mm	Zone of inhibition/mm
Staphylococcus aureus	18	25±2.0	21±2.0
Staphylococcus epidermitis	3	25±1.4	24±4.0
Streptococcus mutans	6	23±0.2	20±.1.2
Streptococcus viridans	12	24±0.2	15±1.0
Pseudomonas aeruginosa	1	18	12
Proteus spp.	1	22	20
Bacillus spp.	1	15	5
Actinobacillus actinomycetemcomitans	2	25±3.0	20±0.5
Candida albicans	2	18	15±1.0

As the primary dental practitioner in the realm of oral hygiene, Prophet Mohammad (peace and blessings be upon him) utilized Siwak as a manducation stick. "I would have commanded them to scrub their teeth with Miswak before every pray if I hadn't found it exhausting for followers or the populace," he claimed.

As a result of chemotherapeutic and antibiotic failures induced by pathogenic microbial infection, several medicinal plants had been screened for antimicrobial activity [18]. In recent years the Miswak extract has also been used in dentistry as an anti-plaque and anti-gingivitis agent. Chewing stems are thought to facilitate salivary secretions, which may aid plaque control and oral cleaning [19]. Our findings revealed that the *Salvadora persica* extract had excellent antibacterial activity against all isolated microorganisms with varying degrees of inhibition zone, and we found that inhibition zone was wide against *Staphylococcus aureus* (25mm), *Staphylococcus epidermidis* (25mm), *Actinobacillus actinomycetemcomitans* (25mm), followed by *Streptococcus viridans* (24mm) *Streptococcus mutans* (23mm), *Proteus spp*. (22mm), *Pseudomonas aeruginosa* (18mm), *Candida spp*.(18mm) *and Bacillus spp*.(15mm) as shown in Table 3. Our findings were consistent with those of others [20-22], They discovered a 0.2-0.3 cm inhibitory zone for the 50% Miswak aqueous extract, with the maximum zone of inhibition for *Staphylococcus aureus* (24mm/2.5l) and the lowest for *Klebsiella pneumoniae.*

On *Pseudomonas aeruginosa, Acinetobacter baumannii*, and *Enterobacter cloacae, Salvadora persica* extract's antibacterial activity resulted in inhibition zones of 20, 18, and 14 respectively [23]. Whereas the maximum exhibition was against *Streptococcus mutans,* and the zones of inhibition for ethanol and hexane extracts were 6–20 mm and 6–16 mm respectively [24]. Others investigated the antibacterial effectiveness of *Salvadora persica* against a variety of oral aerobic and anaerobic microbes [25] and found that the extract had a considerable effect on *Staph. aureus* expansion and a variable effect on various microbe species. The diameter of the inhibition zone in miswak water extract ranged from 15–21mm, with a mean index of 18mm, and *Streptococcus mutans* was the most inhibited bacterium, followed by *Streptococcus salivarius* [5]. *Staph. aureus, Streptococcus mutans E. coli, L. acidophilus, a*nd *P. aeruginosa* strains were found to be susceptible to *Salvadora persica* discs using the disc diffusion assay [2]. In a study, using 50% of Miswak extract the result showed that there was a marked reduction in both *Streptococcus mutant* and *Lactobacillus* [26].

The highest zone of inhibition of *Salvadora persica* extract (22.3mm) against *Streptococcus faecalis* was estimated and in addition, the extract had antifungal activity against *Candida albicans* [27]. According to certain studies, *Streptococcus faecalis* was the most sensitive bacterium to Miswak [28]. The *Salvadora persica* extract had higher inhibitory efficacy against the microorganisms tested, with *Streptococcus* species being the most susceptible. It was also revealed that at 50% concentration, the aqueous extract of Miswak exhibited a 10.5mm zone of inhibition against *Candida albicans*. [16]. The benzyl isothiocyanate found in *Salvadora persica* root chewing sticks have a high killing effect against gram-negative periodontal bacteria

Porphyromonas gingivalis and *Aggregatibacter actinomycetemcomitans* [29]. *In vivo,* testing of *Salvadora persica* revealed significant reductions in the oral cavity counts of *Enterococcus faecalis* and *Candida albicans* [30]. According to another study, stem extracts show antifungal activity against *Candida albicans*. The extracts prepared with 7.5 g of vegetal matter completely inhibited bacteria from the *Streptococcus genus*, such as *Streptococcus mitis* and *Streptococcus faecalis*, as well as bacteria from the *Staphylococcus genus*, such as *Staphylococcus aureus* and *Staphylococcus epidermidis*, and *Lactobacillus casei* [31].

Because of the presence of numerous compounds like sodium chloride and potassium chloride, as well as salvadourea and salvadorine, saponins, tannins, vitamin C, silica, and resin, *Salvadora persica* extracts have antibacterial and cleaning qualities when evaluated as an oral pathogen, it is more efficient than toothpaste against *Staphylococcus aureus* and *Candida albicans.* [18]. Miswak extracts have been discovered to have a variety of biological activity [18]. including substantial antifungal and antibacterial actions, particularly against bacteria that contribute to the formation of dental plaque [20]. It was also discovered that the Miswak sticks remove dental plaque from the interproximal areas of the tooth to the same extent as the other sites [32]. The usefulness of Miswak chewing sticks as a dental care instrument is achieved mechanically by the fibers' ability to reach in between teeth, as well as chemically by the abundance of Phyto-constituents, which are unique in their complexity and biological activity [2]. Miswak possesses anti-cariogenic qualities that prevent plaque formation and, as a result, caries [33]. *Salvadora persica* extract has been discovered to have better antibacterial activity than antibiotics, and it is utilized as a natural source for cleansing the mouth cavity with excellent results. It guards against tooth decay and helps to avoid periodontal disease [23].

Clove oil has been used in dentistry to treat toothaches, periodontitis, and bleeding gums because its components, such as eugenol and caryophyllene, contain great analgesic and antiseptic qualities, preventing the growth of all disease-causing germs while leaving healthy bacteria alone. Clove oil is available in tinctures, lozenges, and mouthwash forms [19]. Cloves are the dried aromatic buds of the *Syzygium aromaticum* tree, which are used as a spice all over the world. Table (3) in the present study showed that clove or *Syzygium aromaticum* had more inhibitory activity against, *Staphylococcus epidermidis* (24mm), *Staphylococcus aureus* (21mm) followed by *Actinobacillus actinomycetemcomitans* (20mm), *Streptococcus mutans* (20mm*), Proteus spp*. (20mm), *Candida spp.* (15mm), *Streptococcus viridans* (15mm), *Pseudomonas aeruginosa* (12mm), *and Bacillus spp*. (5mm). The repressive effect of clove extract against the *Staph. aureus* microorganism has been reported by academic researchers. A previous study demonstrated that the essential oil of *Syzigium aromaticum* dried flower buds had antibacterial activity against *S. aureus, E. coli,* and *P. aeruginosa*. In a study done by Fagere and AL-Magboul, (2016) *Syzigium aromaticum* had strong antibacterial activity against *S. aureus, B. subtilis, E. coli,* and *P. aeruginosa* [34]. Another study found that the essential oil of *S. aromaticum* inhibited *B. subtilis, S. aureus,* and *E. coli* development to varying degrees [35]. An aqueous extract of *Syzygium aromaticum* seeds increased membrane permeability and

oxidative stress in *E. coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus* [36]. When evaluated using the disc diffusion technique, 80.8 % of the isolates were sensitive to clove oil, with the minimum inhibitory concentration of clove oil ranging from 3.125 l/ml to 12.5 l/ml the essential oil of *Syzygium aromaticum* was discovered to have strong antibacterial activity and could be used as an alternative treatment for periodontitis and other orodental infections caused by *A. actinomycetemcomitans*, according to the researchers [37].

The disc diffusion assay revealed that the aqueous extract of clove has good antibacterial activity against *A. actinomycetemcomitans* isolates [38]. The oil of *Syzygium aromaticum* was used to prevent a biotic surface colonized by *Candida* sp. *Syzygium* aromaticum oil contains a high percentage of monoterpenes, eugenol, cinnamic aldehyde, and thymol, which have potent antimicrobial activity but may harm enzymatic cell systems, it also showed antimicrobial activity against *E. coli, Klebsiella pneumoniae, Salmonella paratyphi, Citrobacter spp., Enterobacter cloacae,* and *Staph.* [18].

Salvadora persica (Miswak) and *Syzigium aromaticum* (clove) have antibacterial activity against black-pigmented *Porphyromonas gingivalis, Prevotella intermedia, A. actinomycetemcomitans*, and *Streptococcus mutanus*, and could be used to treat and prevent periodontal disease [9]. As a result, the essential oil of *Syzigium aromaticum* has shown to be effective against a wide range of species. which might be related to the active components in the plant, such as mono and sesquiterpene hydrocarbons, which were found by several coworkers [39, 40]. *Syzigium aromaticum* possesses antimicrobial activity against pathogens causing dental caries and they added that extract was 2-3-fold more effective than common antibiotics and this plant was very safe and had an acceptable taste [38]. Gram-positive bacteria were found to be more active against *Syzigium aromaticum* extract than Gram-negative bacteria [34]. This is most likely owing to Gram-positive bacteria's less complicated cell walls, due to the tiny holes in their cell envelopes, they lack a natural filter action against large molecules. Clove oil was tested against bacterial and fungal strains and was shown to be a powerful antibacterial and antifungal agent, with antibacterial and antifungal activity that was even higher than that of standard antibacterial and antifungal drugs [41]. Clove oil antifungal action was tested on a variety of fungi in a prior study, and it was found to be effective when the concentration of clove oil is increased, and it performs better as an antibacterial and antifungal [42]. Clove's antimicrobial action may cause harm to bacterial cell membranes [43]. The primary component of clove oil, eugenol (2 methoxy-4 allyl phenol), may be responsible for clove's antibacterial and antifungal activities, as well as the high tannin content (10-19 %) [44]. Clove bud oil is used as a treatment in dentistry for minor wounds, as an analgesic in unpleasant situations, as an antiseptic in infectious disorders of the oral cavity and pharynx, and for general hygiene [45]. Because essential oils from fresh and dried roots of *S. persica,* as well as clove oils, are commonly used as antibacterial agents, the use of organic components from plants as an alternative antimicrobial agent is becoming more popular as a result of the development of drug resistance in various pathogens, and oral bacteria are emerging as a greater health threat due to

their involvement in other systemic diseases, in addition to oral diseases such as periodontitis and gingivitis [46].

Conclusion

The study has shown that *Syzigium aromaticum* and *Salvadora persicaextracts have* antibacterial efficacy and their combination has shown a greater effect on oral microorganisms and is recommended to be used for treatment and prevention against oral hygiene. Clove is also known to relieve pain when used to treat toothaches. Because of its antibacterial characteristics, it also aids in the prevention of tooth infections. We concluded that these herbal alternatives or their active constituents could be exploited as potential antimicrobial agents for medicinal purposes.

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References

1. Al-Onayzan AHA, Al mulhem NAH, Al Aboud NSA, Al-Sultan HHS, Ali SI, Sidiq NAH. In vitro efficacy assessment of some toothpaste and Miswak against Streptococcus mutans. Int J Sci Res. 2016; 6:1199-202.

2. Abhary M, Al-Hazmi A-A. Antibacterial activity of Miswak (Salvadora persica L.) extracts on oral hygiene. Journal of Taibah University for Science. 2016;10(4):513-20.

3. Jassoma E, Baeesa L, Sabbagh H. The antiplaque/anticariogenic efficacy of Salvadora persica (Miswak) mouthrinse in comparison to that of chlorhexidine: a systematic review and meta-analysis. BMC Oral Health. 2019;19(1):1-14.

4. Dhaliwal JS, Gambhir RS, Sodhi SK, Shaheed G, Haji DDNBP, Kifli M. Herbs and their use in oral care: A Review. Brunei Darussalam Journal of Health. 2017;7(1):5-17.

5. Al-Sieni AI. The antibacterial activity of traditionally used Salvadora persica L.(miswak) and Commiphora gileadensis (palsam) in Saudi Arabia. African Journal of Traditional, Complementary and Alternative Medicines. 2014;11(1):23-7.

6. Mohammed SG. Comparative study of in vitro antibacterial activity of miswak extracts and different toothpastes. American Journal of Agricultural and Biological Sciences. 2013;8(1):82-8.

7. Gill A, Holley R. Disruption of Escherichia coli, Listeria monocytogenes and Lactobacillus sakei cellular membranes by plant oil aromatics. International journal of food microbiology.

2006;108(1):1-9.

8. binti Bismelah NA. Nor Amiyah binti Bismelah, Dr. Zethy Hanum Mohamed Kassim, Assoc. Professor Dr. Rohana Ahmad, Assoc. Professor Dr. Nor Hadiani Ismail. Journal of Medicinal Plants. 2016;4(2):18-23.

9. **ندى خزعل كاظم المنصوري**. In vitro Antibacterial activity of Plants extracts against Porphyromonas gingivalis, Prevotella intermedia and Aggregatibacter actinomycetemcomitans Streptococcus mutanus, Isolated from Periodontitis Patients in Babylon province, Iraq.

10. Zougagh S, Belghiti A, Rochd T, Zerdani I, Mouslim J. Medicinal and aromatic plants used in traditional treatment of the oral pathology: The ethnobotanical survey in the economic capital Casablanca, Morocco (North Africa). Natural products and bioprospecting. 2019;9(1):35-48.

11. Ahmed ZU, Shafiq K, Wali A, Siddiqui TM, Usman A. Knowledge, attitude and practices of oral health amongst low socioeconomic strata of Sindh. Pakistan Oral & Dental Journal. 2016;36(4).

12. Khan F, Ayub A, Kibria Z. Knowledge, Attitude and practice about oral health among general population of Peshawar. Journal of the Dow University of Health Sciences (JDUHS). 2013;7(3):117-21.

13. Almas K, Al Shwaimi E, Al Shamrani H, Skaug N. The oral hygiene habits among intermediate and secondary school students in Riyadh, Saudi Arabia. Pak Oral Dent J. 2003;23(1):29-34.

14. Casanova-Rosado AJ, Medina-Solís CE, Casanova-Rosado JF, Vallejos-Sánchez AA, Minaya-Sánchez M, Mendoza-Rodríguez M, et al. Tooth brushing frequency in Mexican schoolchildren and associated socio-demographic, socioeconomic, and dental variables. Medical science monitor: international medical journal of experimental and clinical research. 2014; 20:938.

15. Naseem S, Hashmi K, Fasih F, Sharafat S, Khanani R. In vitro evaluation of antimicrobial effect of miswak against common oral pathogens. Pakistan Journal of Medical Sciences. 2014;30(2):398.

16. Al-Bayati FA, Sulaiman KD. In vitro antimicrobial activity of Salvadora persica L. extracts against some isolated oral pathogens in Iraq. Turkish Journal of Biology. 2008;32(1):57-62.

17. Sher H, Al-yemeni MN, Wijaya L. Ethnobotanical and antibacterial potential of Salvadora persica l: a well-known medicinal plant in Arab and Unani system of medicine. Journal of Medicinal Plants Research. 2011;5(7):1224-122.

18. Gomaa M, Almaghrabi O, Elshoura A, Soliman A, Gharieb M. Novel mixture of chloroxylenol and copper alters Candida albicans biofilm formation, biochemical characteristics, and morphological features. Journal of Taibah University for Science. 2020;14(1):889-95.

19. Dhaliwal JS, Gambhir RS, Sodhi SK, Shaheed G, Binti D, HajiPG KM. Herbs and their use in oral care: A Review. Brunei Darussalam Journal of Health. 2017;7(1):5-17.

20. Ababneh H. The effect of the extract of the miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. International Dental Journal. 1995;45(3):218-22.

21. Almas K, Al-Bagieh N, Akpata E. In vitro antimicrobial effects of extracts of freshly cut and 1-month-old miswak (chewing stick). Biomedical Letters (United Kingdom). 1997.

22. Almas K. The antimicrobial effects of extracts of Azadirachta indica (Neem) and Salvadora persica (Arak) chewing sticks. Indian journal of dental research: official publication of Indian Society for Dental Research. 1999;10(1):23-6.

23. Mammad Z, Hsaine S, Djassinra T, Ounine K. The antibacterial and antioxidant effect of Salvadora persica on antibiotic resistant strains. American Journal of Plant Sciences. 2018;9(07):1478.

24. Balto H, Al-Sanie I, Al-Beshri S, Aldrees A. Effectiveness of Salvadora persica extracts against common oral pathogens. The Saudi dental journal. 2017;29(1):1-6.

25. Burt SA, Reinders RD. Antibacterial activity of selected plant essential oils against Escherichia coli O157: H7. Letters in applied microbiology. 2003;36(3):162-7.

26. Bhat PK, Kumar A, Sarkar S. A Comparative Clinical Study to Assess the Immediate Antimicrobial Effect of Miswak and Toothbrush on Cariogenic Bacteria. World Applied Sciences Journal. 2011;15(6):899-903.

27. Haque MM, Alsareii SA. A review of the therapeutic effects of using miswak (Salvadora Persica) on oral health. Saudi medical journal. 2015;36(5):530.

28. Ezoddini-Ardakani F. Efficacy of Miswak (salvadora persica) in preventing dental caries. Health. 2010;2(05):499.

29. Sofrata A, Santangelo EM, Azeem M, Borg-Karlson A-K, Gustafsson A, Pütsep K. Benzyl isothiocyanate, a major component from the roots of Salvadora persica is highly active against Gram-negative bacteria. PLoS One. 2011;6(8): e23045.

30. Shafiei Bafti L, Rad M, Salehi Soormaghi M, Rezaei M. An in vivo evaluation of

antimicrobial effects of Persica herbal mouthwash on Candida albicans and Enterococcus faecalis. Journal of Oral Health and Oral Epidemiology. 2013;2(2):64-9.

31. Aumeeruddy MZ, Zengin G, Mahomoodally MF. A review of the traditional and modern uses of Salvadora persica L.(Miswak): Toothbrush tree of Prophet Muhammad. Journal of Ethnopharmacology. 2018; 213:409-44.

32. Almas AK, Almas K. Miswak (salvadora persica chewing stick) and its role in oral health; an update. JPDA. 2013;22(04):255.

33. Halawany HS. A review on miswak (Salvadora persica) and its effect on various aspects of oral health. The Saudi dental journal. 2012;24(2):63-9.

34. Fagere ZO, Al Magbou A. Antibacterial activity of clove oil against some microorganisms at Khartoum state, Sudan. Adv Med Plant Res. 2016;4(4):122-8.

35. Javed S, Mushtaq S, Khokhar I, Ahmad R, Haider MS. Comparative antimicrobial activity of clove and fennel essential oils against foodborne pathogenic fungi and food spoilage bacteria. African Journal of Biotechnology. 2012;11(94):16065-70.

36. Ajiboye T, Mohammed A, Bello S, Yusuf I, Ibitoye O, Muritala H, et al. Antibacterial activity of Syzygium aromaticum seed: Studies on oxidative stress biomarkers and membrane permeability. Microbial Pathogenesis. 2016; 95:208-15.

37. Karicheri R, Antony B. Antibacterial Activity of Essential Oil of Syzygium Aromaticum (L.) Merr.Perry (Cloves) Against Clinical Isolates of Aggregatibacter Actinomycetemcomitans. 2015.

38. Pathak A, Sardar A, Kadam V, Rekadwad B, Karuppayil SM. Efficacy of some medicinal plants against human dental pathogens. 2012.

39. Rodríguez Luis O, Sánchez Casas RM, Verde Star MJ, Núñez A, Ríos R, Chávez A. Obtaining the essential oil of Syzygium aromaticum, identification of eugenol and its effect on Streptococcus mutans. Journal of oral research. 2014;3(4):218-24.

40. Nana WL, Eke P, Fokom R, Bakanrga-Via I, Begoude D, Tchana T, et al. Antimicrobial activity of Syzygium aromaticum and Zanthoxylum xanthoxyloides essential oils against Phytophthora megakarya. Journal of Phytopathology. 2015;163(7-8):632-41.

41. Aneja KR, Joshi R. Antimicrobial activity of Syzygium aromaticum and its bud oil against dental cares causing microorganisms. Ethnobotanical Leaflets. 2010; 14:960-75.

42. Verma S, Karkun A, Siddiqui HN. Comparative study of clove oil against bacteria and

fungal species. International Journal of Clinical Biochemistry and Research. 2015;2(2):73-6.

43. Pulikottil S, Nath S. Potential of clove of Syzygium aromaticum in development of a therapeutic agent for periodontal disease: A review. South African Dental Journal. 2015;70(3):108-15.

44. Abdullah BH, Hatem SF, Jumaa W. A comparative study of the antibacterial activity of clove and rosemary essential oils on multidrug resistant bacteria. Pharmaceutical and Biosciences Journal. 2015:18-22.

45. Shah ST, Gathece LW, Simiyu NB, Mulli TK. Effect of homemade dental powder on population of streptococcus mutans in vitro. 2016.

46. Khan M, Alkhathlan HZ, Khan ST. Antibiotic and antibiofilm activities of Salvadora persica L. essential oils against Streptococcus mutans: A detailed comparative study with chlorhexidine digluconate. Pathogens. 2020;9(1):66.