Antimicrobial Activity of *Syzigium Aromaticum* (Clove) and *Salvadora Persica* (Miswak) Against Dental Plague Pathogens

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Abstract*—Some periodontal bacteria on tooth plaque were shown to be sensitive to extracts from *Syzigium aromaticum* and *Salvadora persica*. Our study was an assessment of the antimicrobial effect of *Syzigium aromaticum* and *Salvadora persica* extracts against dental plague pathogens. Thirty-five 35 oral swabs were taken from the patients who had the dental plague and isolated microorganisms were identified by standard bacteriological methods. *Syzigium aromaticum* and *Salvadora persica* extracts were tested against dental plaque microorganisms on Muller Hinton agar, antimicrobial activity of two extracts were done by paper disks-diffusion technique at 50% concentration and characterized by inhibition zones. Forty-six (46) microorganisms were isolated from 35 dental plague samples,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 aeruginosa*., *Proteus spp., Bacillus* spp., 2 isolates were *Actinobacillus actinomycetemcomitans* and 2 isolates were *Candida albicans.* The zones of inhibition for two different extracts were measured. According to the findings, *Syzigium aromaticum* and *Salvadora persica* have antibacterial and antifungal effects against gram-negative and gram-positive bacteria, as well as fungi. Our study has shown that *Syzigium aromaticum* and *Salvadora persica* extracts have antimicrobial efficacy, and concluded that the extracts have shown a greater effect on oral microorganisms and are recommended to be used for treatment and prevention against oral hygiene.

Index Terms*— Antimicrobial activity, dental plague, *Candida albicans, Syzigium aromaticum, Salvadora persica.

I. 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 are 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, 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* have 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 a spice all over the world and their oil has two major components, eugenol, and β-caryophyllene. The eugenol has bactericidal effects which cause disruption of the cytoplasmic membrane of bacterial cells so that extracts from *Syzygium aromaticum* have been shown to be efficient 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's oil shows 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 plague germs.

II. MATERIALS AND METHODS

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

Materials

In Erbil City, Kurdistan, Iraq, chewing sticks *Salvadora persica* and *Syzygium aromaticum* were purchased in a local market. Thirty-five (35) 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, use of toothbrushes.

Culture Media

All samples were cultured in Blood agar (oxide); MacConkey agar (oxide), Trypticase soy agar plates supplemented with

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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 a sensitivity test. The isolated microorganisms were recognized by colony morphology and by using API 20 E and API Staph.

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 anaerobic incubated 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 colony morphology on the media that are freshly isolated from patients has fimbriae and inner star-shaped.

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* was cleansed and rinsed with sterile distilled water before being shade dried and pulverized using a mechanical grinder. Both plant powders were weighed into a 10gm quantity with 100 mL of sterile deionized distilled water were mixed 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 sieved through a Whatman No. 1 filler paper, and extracts of both plants were prepared at a 50% concentration and stored in sterilized screw-capped vials in the refrigerator until needed.

Preparation of Microorganisms

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

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 microorganism 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 for 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.

III. RESULTS

The gender and age distribution of patients were studied. (table1). A total of 35 patients, 48.6% were female and 51.4% were male. Our findings revealed that the age groups (50-59) and (60-69) years old had the most prevalence of dental plaque, according to our findings, with males being more sensitive than females, these results agree with other studies 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 may cause to tooth loss. These findings suggested that a lack of understanding of oral hygiene guidelines, a crucial essential for defensive, conservative, and prosthetic dental treatments, or risk factors in these age groups, such as poor hygiene and smoking, could be contributing factors [9, 10].

TABLE 1 Patients' age and gender distribution

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 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 tooth brush to brush their teeth [11]. In 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 and13.6% females) of subjects were never cleaned their teeth. Only 36 (19%) of females and 30 (27.2%) of males have 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 of cleanliness creates, knowledge of the presence of certain cleaning instruments, education levels, and socioeconomic position [13]. Individuals of lower socio-economic status have fewer incomes to address oral health issues: less free time, less money to buy toothbrushes and toothpaste, resulting in poor oral hygiene because of a lack of proper dental knowledge, leading to an increase in the prevalence of dental caries globally, with treatment being expensive [14, 15].

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TABLE 2 Distribution Tooth brushing/ day in relation to 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 plague samples in 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]. 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 extracts 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 infection by pathogenic microbes, several medicinal plants had been screened for antimicrobial activity [18]. In the recent years the Miswak extract has also used in dentistry as anti-plaque and anti-gingivitis agents. Chewing stems is 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% aqueous Miswak extract, with the highest zone of inhibition for *Staphylococcus aureus* (24m/2.5l) and the lowest zone for *Klebsiella pneumoniae* (13mm/2.5l).

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, respectively, were 6–20 mm and 6–16 mm [24]. Others investigated the antibacterial effectiveness of *Salvadora persica* against a variety of aerobic and anaerobic oral 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]. *Staphylococcus 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, by using 50% of miswak extract the result showed that there was marked reduction in both *Streptococcus mutant* and *Lactobacillus* [26].

The highest zone of inhibition of *Salvadora persica* extract (22.3 mm) 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 aqueous *Salvadora persica* extract showed higher inhibitory efficacy against the tested microorganisms, with *Streptococcus* species being the most susceptible. It was also revealed that at 50% concentration, the aqueous miswak extract exhibited a 10.5mm zone of inhibition against *Candida albicans* [16]. The benzyl isothiocyanate found in *Salvadora persica* root chewing sticks has a high killing efficiency 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, antifungal activity against *Candida albicans* showed by stem extracts. 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].

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Because of the presence of numerous compounds like sodium chloride and potassium chloride, as well as salvadorine and salvadourea, vitamin C, saponins, tannins, silica, and resin, *Salvadora persica* extracts have antibacterial and cleaning properties when evaluated as an oral pathogen, it is more efficient than tooth paste against *Staphylococcus aureus* and *Candida albicans* [18]. Miswak extracts have been discovered to have a variety of biological actions [18], including substantial antifungal and antibacterial activities, 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 complication and biological vigor [2]. Miswak possesses anticariogenic 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, have antiseptic properties and excellent analgesic, preventing the growth of all germs causing disease while leaving normal flora bacteria alone. Clove oil is available in tincture, lozenges, and mouth wash 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 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 essential oil of *S. aromaticum* dried flower buds had bactericidal activity against *S. aureus*, *E. coli* and *P. aeruginosa*. In a study done by Fagere and AL-Magboul, (2016) *S. aromaticum* had strong antibacterial activity against *S. aureus, B. subtilis*, *E. coli* and *P. aeruginosa* [34]. Another study found that the vital oil of *S. aromaticum* inhibited *B. subtilis, S. aureus,* and *E. coli* development to varying degrees [35]. An aqueous *Syzygium aromaticum* seed extract increased membrane permeability and oxidative stress in *E. coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus* [36]. According to the findings, 80.8% of the isolates were sensitive to clove oil when tested using the disc diffusion technique, with the lowest inhibitory concentration of clove oil ranging from 3.125 l/ml to 12.5 l/ml. and concluded that the essential oil of *Syzygium aromaticum* was found to have strong antibacterial activity and could be utilized as an alternative treatment for periodontitis and other orodental infections caused by *A.actinomycetemcomitans* [37].

The disc diffusion assay revealed that 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 proportion of monoterpenes, cinnamic aldehyde, eugenol, and thymol, which have potent antimicrobial activity but may harm enzymatic cell systems*,* it also displayed antimicrobial activity against *E. coli, Klebsiella pneumoniae, Salmonella paratyphi, Citrobacter spp., Enterobacter cloacae,* and *Staph. aureus* [18].

Salvadora persica (Miswak) and *Salvadora aromaticum* (clove) have antibacterial activity against black-pigmented *P. gingivalis, P. intermedia, A. actinomycetemcomitans,* and *Streptococcus mutanus*, and could be used to treat and prevent periodontal disease [9]. As a result, the fundamental oil of *S. 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* possess 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 have acceptable taste [38]. Gram positive bacteria were found to be less tolerant against *S. aromaticum* extract than Gram negative bacteria. [34]. This is most likely owing to Gram positive bacteria's less complicated cell walls, due of 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 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's 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 antibacterial agent is becoming more popular as a result of the expansion of drug resistance in various pathogens, and oral bacteria are emerging as a greater health threat due to their participation in other systemic diseases, as well as oral diseases such as gingivitis and periodontitis [46].

CONCLUSIONS

Our study has shown that *Syzigium aromaticum* and *Salvadora persicaextracts have* antibacterial efficacy and their combination have shown greater effect on oral microorganisms and is recommended to be used for treatment and prevention

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against oral hygiene. Clove is also known to relieve pain when used to treat toothaches. Because of its antiseptic and antibacterial efficiency, it also aids in the prevention of tooth infection. We concluded that these herbal alternatives or its active constituents could be exploited as potential antimicrobial agents for medicinal purposes.

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