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MASTER'S THESIS

**THE EFFECT OF MISWAK (*SALVADORA PERSICA*) ON THE
ORAL HEALTH OF ORTHODONTIC PATIENTS**

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Hereby I acknowledge that the thesis work is mine; I had no unethical behaviors from planning up to the writing of the thesis, I obtained all information on this thesis within the academic and ethical rules, I have added all the information and interpretation obtained from other reference and authors to the reference list, I had no behaviors that violate the patent and copyrights during the writing and thesis study.



ZAKARIYA ABUHARFIL

DEDICATION

This thesis is dedicated to my beloved parents, Khadeejah and Prof. Dr. Nizar. Their endless love, support, and encouragement have been the foundation of my journey, and I could not have completed my graduate studies without them. I deeply love and appreciate all that they have done for me.

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LIST OF ABBREVIATIONS AND SYMBOLS

WHO	: World Health Organization
BITC	: Benzyl isothiocyanate
C. albicans	: Candida albicans
GPC	: Gram-positive cocci
MDR	: Multiple drug resistance
MIC	: Minimum inhibitory concentration
MRS	: Man, Rogosa, and Sharpe
NaF	: Sodium fluoride
S. aureus	: Staphylococcus
S. persica	: Salvadora persica
TM	: Temporomandibular dysfunction
WSLs	: White spot lesions
MG	: Miswak Group
TG	: Toothpaste Group

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1. ABSTRACT

THE EFFECT OF MISWAK (*SALVADORA PERSICA*) ON THE ORAL HEALTH OF ORTHODONTIC PATIENTS

Good oral health plays a vital role in overall well-being and quality of life. Numerous chronic and systemic diseases have been linked to poor oral hygiene, highlighting the importance of effective preventive and therapeutic strategies. With the rising prevalence of oral diseases, the global demand for alternative, safe, and cost-effective oral health solutions has increased. Maintaining oral hygiene primarily relies on mechanical and chemical methods, with toothbrushes and fluoride-based dentifrices being the most widely used tools for plaque removal and dental care.

Fluoride toothpaste has been extensively used to prevent dental caries and enhance enamel remineralization. While its benefits are well-documented, concerns have arisen regarding excessive fluoride exposure, which has been associated with dental fluorosis, skeletal fluorosis, gastrointestinal disturbances, neurodevelopmental effects, and thyroid dysfunction. Given these potential risks, there is increasing interest in natural alternatives that provide effective oral hygiene without the adverse effects of fluoride overexposure.

For centuries, plants have been utilized for dental care, with many traditional communities still relying on herbal and natural methods to maintain oral hygiene. One such method is Miswak, an Arabic term meaning "tooth-cleaning stick," derived from *Salvadora persica*. This plant has a wide geographic distribution and has been historically used by ancient civilizations, particularly in the Arab world, to whiten and polish teeth. Scientific research has confirmed that Miswak possesses antibacterial, antifungal, antiviral, anti-cariogenic, and anti-plaque properties, making it a potential alternative to fluoride toothpaste.

In addition to its mechanical cleaning action, Miswak exhibits antioxidant, analgesic, and anti-inflammatory properties, contributing to its potential benefits in oral health maintenance. Studies have shown that its use influences the composition of saliva, supporting a healthier oral microbiome. Furthermore, clinical research has

demonstrated that the mechanical and chemical efficacy of Miswak is comparable to, and in some cases superior to, that of traditional toothbrushes and fluoride-based products.

This study explores the effectiveness of Miswak (*Salvadora persica*) in maintaining oral health among orthodontic patients. Given the unique challenges orthodontic patients face in plaque removal and gingival health maintenance, this research aims to assess Miswak's impact on oral hygiene compared to fluoride-based products, evaluating its antimicrobial efficiency, plaque reduction capabilities, and overall safety. Through this investigation, we seek to determine whether Miswak can serve as a viable and effective alternative for orthodontic patients in maintaining optimal oral health.

Keywords:

Alternative dentistry, anti-cariogenic, anti-inflammatory properties, antimicrobial properties, fluoride toothpaste, gingival health, miswak, oral health, orthodontic patients, periodontal health, plaque reduction, saliva composition, *salvadora persica*, tooth remineralization.

2. ÖZET

MİSVAKIN (SALVADORA PERSICA) ORTODONTİK HASTALARIN AĞIZ SAĞLIĞINA ETKİSİ

Bu çalışma, Misvak'ın (*Salvadora persica*) ortodontik hastalar için bir ağız hijyen aracı olarak etkinliğini araştırmaktadır. Sabit ortodontik apareylerin ağız hijyenini sürdürmede yarattığı zorluklar göz önünde bulundurularak, araştırma Misvak'ın antimikrobiyal özelliklerini, bakteriyel popülasyonlar (*Streptococcus mutans* ve *Lactobacillus*) üzerindeki etkisini, plak azalması, diş eti sağlığı ve periodontal durum ile olan ilişkisini incelemektedir. Çalışmanın amacı, Misvak'ın geleneksel florür içeren ağız bakım ürünleriyle karşılaştırmasını yapmak ve doğal, ekonomik bir alternatif olarak potansiyelini değerlendirmektir. Ortodontik tedavi, ağız ortamında değişikliklere yol açarak plak birikimini artırır, mikrobiyal büyümeye neden olur ve periodontal sorunlara yol açabilir. Geleneksel diş bakımında yaygın olarak kullanılan Misvak, antibakteriyel, antikaryojenik ve anti inflamatuvar özellikleriyle bilinmektedir. Bu çalışma, misvak (*Salvadora persica*) tozunun doğal bir diş macununa eklenmesinin ortodontik hastaların ağız sağlığı üzerindeki etkilerini 2 ay boyunca incelemiştir. Bulgular, misvağın bakteriyel yükü, plak birikimini ve diş eti iltihabını azaltarak periodontal sağlığı iyileştirdiğini göstermiştir. Özellikle ağız hijyenini korumakta zorluk yaşayan ortodontik hastalarda olumlu etkiler gözlemlenmiştir. Misvak, antimikrobiyal, antiinflamatuvar ve plak azaltıcı özellikleriyle geleneksel diş bakımına etkili bir alternatif sunmaktadır. Klinik olarak, modern diş bakımına erişimi kısıtlı bireyler için faydalı bir seçenek olabilir. Ayrıca, doğal bileşenleri sayesinde florürlü ürünlere sürdürülebilir bir alternatif olarak değerlendirilmektedir.

Sonuç olarak, Misvak, ortodontik hastalar için etkili bir ağız hijyeni aracı olarak potansiyel göstermektedir. Doğal antibakteriyel özellikleri, kullanım kolaylığı ve kültürel kabulü, onu geleneksel florür bazlı ağız bakım ürünlerine umut verici bir alternatif haline getirmektedir. Ortodontik tedavi sonuçları üzerindeki uzun vadeli etkisini araştırmak için daha fazla çalışma önerilmektedir.

Anahtar Kelimeler: Antimikrobiyal özellikler, dental plak kontrolü, florürsüz ağız bakımı, diş eti sağlığı, misvak, ortodontik hastalar, periodontal sağlık, *salvadora persica*, *Streptococcus mutans*

3. INTRODUCTION AND PURPOSE

3.1. Oral Health

Maintaining good oral health is pivotal to enhancing overall quality of life and well-being, affecting not only dental health but also systemic health, nutrition, sleep, and social engagements. Numerous chronic and systemic illnesses have been linked to inadequate oral health, highlighting the necessity for effective oral hygiene routines to safeguard both dental and general health. As the prevalence of oral diseases rises globally, there is a growing need for alternative preventative and therapeutic approaches, alongside safe, efficient, and cost-effective products. The upkeep of oral health can largely be accomplished through both mechanical and chemical methods, with toothbrushes and toothpaste being the most prevalent instruments for tooth cleaning. The current design of the toothbrush has progressed from primitive tools like chewing sticks, which were utilized by Babylonians around 3500 BC, and toothpicks referenced in ancient Greek and Roman texts (1,2).

Poor oral health is closely associated with systemic issues including cardiovascular disease and diabetes, further reinforcing the significance of robust oral hygiene practices in improving quality of life. While toothbrushes and toothpaste are the most commonly used instruments for oral care, age-old techniques such as miswak, derived from *Salvadora persica*, remain prevalent in various cultures (3). Miswak has been shown to be efficient in plaque removal and minimizing gingival inflammation, attributed to its bioactive components (4).

Herbal remedies such as miswak have been employed for centuries, noting not only their effectiveness but also their cultural significance in areas without modern dental care (5). The use of miswak, in particular, is deeply ingrained in Islamic traditions, underscoring its ties to religious practices (6).

In the field of orthodontics, which encounters unique hurdles in maintaining oral care, miswak has been highlighted for its ability to control plaque accumulation and avert dental caries in patients with fixed orthodontic appliances (7,8). Likewise,

studies have demonstrated that the use of antimicrobial agents like miswak can reduce bacterial proliferation linked to orthodontic bands, thereby enhancing oral health (8,9).

The antimicrobial properties of *Salvadora persica* are extensively documented, confirming its effectiveness in lowering levels of cariogenic bacteria such as *Streptococcus mutans* (8,10). Further supporting the health benefits of miswak, significant antibacterial activity in *Salvadora persica* extracts against oral pathogens has been demonstrated, advancing its practical uses in dental hygiene (6,11).

Benzyl isothiocyanate (BITC), an important compound of *Salvadora persica*, has demonstrated formidable antibacterial prowess. Investigations have acknowledged its potency in targeting periodontal bacteria, positioning miswak as a natural alternative for periodontal therapy (12,13). Recent research further affirms the significance of traditional methods, revealing that brushes and chewing sticks made from *Salvadora persica* rival standard toothbrushes in effectively managing plaque and fostering gum health (14).

To summarize, a systematic review concluded that miswak offers enhanced anti-plaque and anti-gingivitis benefits compared to typical toothbrushes, solidifying its status as a practical and efficient tool for oral hygiene (15).

3.2. Using Of Chewing Sticks And Miswak For Oral Hygiene

Chewing sticks are made from a variety of plant species and they are usually used to clean teeth in Asia, Africa, South America, and the Middle East. Western travelers and discoverers described the use of caustic nests from men and women in the Sahara and Sudan regions (6).

Residents in these areas will eagerly clean their teeth when they have the opportunity to sit for a social meeting. Chewing poles are known by different names in different cultures: "arak" or "miswak" in Arabic, "koyoji" in Japanese, "Qesam" in Hebrew, "Qisa" in Aramaic, "Arami" in Arami and "mastic" Latin (16). They are popular for their modern, availability, low cost, simplicity, religious and/or traditional relationships between *Kaustocken*. Chew can play a role in promoting oral hygiene, and further evaluation of its effectiveness has been reported as described in the World

Health Organization 2000 (WHO) consensus report (17) on oral hygiene. "Miswak" (various Arabic dialects and countries synonyms include "Mizuwak, "mankuwak", "mizuwaki", "meswaki", "muswaki", "sewak", "sewak", "siwak", "swak", "swak", "swak", "swak", "siwaki") is an Arabic word meaning to heal the teeth (18).

The spread of Islamic culture had a major impact on the spread and use of Miswak, originally a pre-Islamic practice in various parts of the world. Of at least 182 plant species suitable for the production of toothbrushes, most are harvested from *Salvadora persica*. The roots, branches, and stems of this plant were used for oral hygiene, with small *Salvadora persica* sticks being used as toothpicks (19).

Salvadora persica is a broad geographical area that runs through Pakistan, Iran, Iraq, Saudi Arabia, and Egypt, from the eastern states of Rajasthan (India), Nepal, and Malaysia to Mauritania in the west, North Africa, Sudan, Egypt, and the Central Region. It has a distribution from Africa to southwest Africa (20). In the field of oral hygiene, taking into account the historical importance of the use of *Salvadora persica* Miswak, in the current review, this practice contributes to dentistry and explains the contributions from the latest literature and the major literature. It should remind readers of their contributions and the aspects of the impact on oral health, including their disadvantages.

3.3. Chemical Analysis of Miswak

Chemical compositional analysis of *miswak* has shown the presence of B-sitosterol and M-ancient acids, chlorides, *Salvadora*, and plaster. Organic compounds such as pyrrolidine, pyrrole, and piperidine derivatives. Glycosides such as flavonoids including salvadoside, salvadoside, kaempferol, quercetin, quercetin rutin, and quercetin-glucoside.

The roots and bark of the *Salvadora persica* tree are composed of 27% ash, a high ratio of alkaloids such as salvadorine and trimethylamine, chlorides, and fluorides. Moderate concentrations of silica, sulfur, and vitamin C are present, along with small amounts of tannins, saponins, flavonoids, and sterols (21). Large quantities

of sodium and potassium chloride were discovered, along with significant amounts of calcium and phosphorus released into the water (22).

It has been found that the repeated chewing method of *Salvadora persica* Miswak releases fresh sap that may have anti-cariogenic properties (23). However, another study found that when *Salvadora persica* Miswak was immersed in water, a negligible amount (0.07 Lg/ml) of fluoride was released (18).

Farooqi and Srivastava (1968) separated benzyl isothiocyanate (BITC) from the *Salvadora persica* root, and BITC is a final product derived from the enzymatic hydrolysis of glucosinolates present in plants. BITC is a chemical treatment that assumes similarity and other genotoxic compounds that reach or respond to target regions of treated tissues. A concentration of 133.3 LG/mL of BITC was indicated by viral acid activity against herpes simplex virus (24).

Furthermore, BITC has extensive bacterial activity and inhibits *Salvadora persica* growth and acid production. Other elements of *Salvadora persica* also have a beneficial effect on oral health. The sulfur content of root ash of *Salvadora persica* was up to 4.73% (6).

Furthermore, sulfur in *Salvadora persica* has a bacterial effect, and vitamin C helps in tissue healing and repair (25). Stain acid acts as an abrasive and helps to remove spots on the surface of teeth. The aspiration effect of tannins helps reduce clinically detectable gingivitis. Tannins were found to inhibit the effect of glucosyltransferases, thereby reducing plaque and gingivitis. The resin from Miswak can form layers in enamel that protects against tooth decay (26).

Salvadora persica Alkaloid present in *Salvadora persica* Miswak has bacterial suicide and can stimulate the gums (21). The slightly bitter taste of *Salvadora persica* essential oils stimulates saliva flow (6).

3.4. Aim Of The Study

This study's objective is to thoroughly investigate the impact of Miswak (*Salvadora persica*) on the oral health of patients undergoing orthodontic treatment. This includes an in-depth analysis of its antimicrobial properties, focusing on the fluctuation of bacterial populations such as *Streptococcus mutans* and *Lactobacillus*, and its effect on gingival and periodontal health. Additionally, the study seeks to assess the potential of Miswak as a natural and economical alternative to conventional oral hygiene methods, evaluating its ability to reduce dental plaque, maintain periodontal status, and promote overall oral hygiene during orthodontic treatment. By addressing these aspects, the research aims to contribute to the understanding of Miswak's role in preventing complications commonly associated with fixed orthodontic appliances and enhancing oral health.

Reiterating, this study seeks to investigate the impact and significance of *Salvadora persica* extracts on oral health during orthodontic treatment, with particular emphasis on specific bacterial populations and periodontal health. The objectives of the research are outlined below:

- To analyze the impact of Miswak (*Salvadora persica*) on the gingival and periodontal health of Miswak using group versus ordinary commercially available toothpaste using group. From this point on word, we will record these groups as **Miswak Group (MG)** and **Toothpaste Group (TG)**.
- To evaluate the influence of Miswak on the total bacterial load, including both aerobic and facultative anaerobic bacteria, during orthodontic treatment. Likewise, we carried out the same battery of tests for the TG.
- To determine the specific effects of Miswak on *Streptococcus mutans* and *Lactobacillus* populations within the oral environment. Likewise, the same was conducted on TG.
- To compare the efficacy of Miswak with conventional oral hygiene methods in reducing dental plaque and improving overall oral hygiene outcomes.
- To investigate the potential benefits of integrating Miswak as a natural, cost-effective adjunct in orthodontic care practices.

○ 3.5. Research Hypotheses

- Null Hypothesis (HQ):
 - HQ1: Miswak (*Salvadora persica*) does not have a significant effect on the oral health of orthodontic patients.
 - HQ2: Miswak (*Salvadora persica*) does not significantly reduce the bacterial populations, including *Streptococcus mutans* and *Lactobacillus*, in orthodontic patients.
 - HQ3: Miswak (*Salvadora persica*) does not improve gingival and periodontal health outcomes in orthodontic patients.
- Alternative Hypothesis (HA):
 - HA1: Miswak (*Salvadora persica*) significantly improves the oral health of orthodontic patients.
 - HA2: Miswak (*Salvadora persica*) effectively reduces the levels of *Streptococcus mutans*, *Lactobacillus*, and other pathogenic bacteria in orthodontic patients.
 - HA3: Miswak (*Salvadora persica*) enhances gingival and periodontal health, leading to reduced plaque accumulation and inflammation in orthodontic patients.

4. GENERAL INFORMATION

4.1. Changes to Patients' Oral Environments During Orthodontic Treatment

In dentistry, orthodontic treatment is one specialty that, like many others in the field, may have unfavorable outcomes from treatment implementation. Both the patient and the practitioner may be responsible for these outcomes. Some of these effects—like root resorption—are not entirely understood, while others are linked to orthodontic therapy without any supporting data. It is critical to consider risk factors before beginning treatment. These negative consequences include root resorption, discomfort, pulpal alterations, periodontal disease, decalcification, temporomandibular dysfunction (TMD) (8,27)

Dental enamel and the oral environment are in balance under normal conditions, and saliva has all the components needed for the formation of hydroxyapatite crystals. Demineralization and remineralization are ongoing processes in this natural condition (28).

This natural equilibrium is upset by orthodontic appliances, but when the appliance is removed, a favorable balance between demineralization and remineralization is restored (29), which may allow lesions to heal naturally. As long as other etiological factors are favorable, natural repair will take place in the presence of sufficient levels of calcium, phosphate, and fluoride ions in the saliva and plaque. This natural healing, however, has been demonstrated to be restricted (27).

It is claimed that fixed appliances with self-ligating brackets are easier to clean and accumulate less plaque. On this point, there is disagreement, though. When comparing Self-ligating brackets (SLB) to conventional brackets (CB), practitioners who reported longer appointment intervals (10 weeks or more) were more likely to favor SLB for the majority of treatment factors, such as faster initial treatment progress, better oral hygiene, shorter adjustment appointments, and overall shorter treatment time. (30).

Since SL brackets eliminate the need for elastomeric ligatures, they are also thought to improve oral hygiene in patients receiving fixed appliance therapy by

reducing plaque retention. Less retention of oral bacteria, including streptococci, and fewer bacteria in plaque were observed on tooth surfaces bonded using SL brackets as opposed to conventional brackets in two trials that examined plaque formation and bacteria around the various modalities of ligation (31).

Malocclusions are linked to several issues and rank as the third most prevalent oral health issue. Although orthodontic therapy frequently resolves these difficulties or at least stops them from getting worse, there is a chance that it could damage teeth and periodontal tissues. For instance, it could be challenging to maintain good dental hygiene while receiving therapy, which could result in gingival irritation and plaque buildup. Few things are known about periodontal inflammation that causes occult blood in saliva and the acidity of dental plaque, but it has been demonstrated that orthodontic treatment alters the oral environment by increasing the concentration of bacteria and changing buffer capacity, pH acidity, and salivary flow rate (8).

When patients are receiving orthodontic treatment with fixed appliances, it is critical to recognize the changes in their oral environment. In certain situations that require lengthy therapy, the clinicians are dedicated to maintaining the patient's dental health. *Streptococcus mutans* and *Lactobacillus* spp. counts increased during the six-month follow-up period, suggesting that the orthodontic group's oral microbiota evolved over time in comparison to the control group (32).

Tyler and Goh conducted a randomized controlled experiment to determine whether using a Waterpik® in addition to a manual toothbrush is better for oral hygiene maintenance than using a manual toothbrush alone in patients with fixed orthodontic equipment. According to the study, patients with fixed orthodontic appliances do not statistically or clinically benefit from using a Waterpik® in addition to a manual toothbrush. However, any conclusions should be considered cautiously because the trial's data is insufficient, and the analysis was conducted on a small sample size (33).

In a different study, the efficiency of interdental flossing and water jet flossing in preserving oral hygiene in individuals receiving orthodontic treatment with fixed appliances was compared. The study's purpose is to evaluate how well water jet and

interdental flossing maintain oral hygiene in patients receiving orthodontic treatment with fixed appliances.

4.2. Orthodontic treatment-related pain

One typical side effect of orthodontic therapy is pain and discomfort. According to earlier research, 70–95% of orthodontic patients report feeling discomfort (34). Patients may feel pain from gum and periodontal disease in addition to the pain and discomfort of therapy. The discomfort linked to orthodontic therapy is marked by pressure, tension, or soreness in the teeth. The discomfort in the anterior teeth surpasses that in the posterior teeth (35). Discomfort is reported to begin four hours post-placement of separators or orthodontic wire, with the peak discomfort occurring on the second day of treatment (36). The discomfort often endures for seven days. The clinical expectation of utilizing permanent equipment increases the likelihood of pain and suffering (37).

4.3. Fixed orthodontic treatment and periodontal disease

Gingivitis, alveolar bone loss (periodontitis), and the loss of associated gingival support are examples of periodontal disorders. Numerous factors, including host resistance, systemic illnesses, and the amount and makeup of tooth plaque, affect the periodontal response to orthodontic appliances. Smoking is one lifestyle choice that might compromise periodontal support. It is commonly known that uncontrolled diabetes negatively affects periodontal support. People with uncontrolled diabetes should not receive orthodontic therapy (38). Bacteria present in dental plaque are considered the primary causative agents of periodontal disease. It is known that orthodontic treatment with fixed appliances increases the volume of dental plaque. However, fixed orthodontic appliances are believed to cause a shift in the type of bacteria. Therefore, fixed orthodontic treatment may result in localized gingivitis, which rarely progresses to periodontitis (39).

The factor that determines the condition of the periodontium during orthodontic treatment is the level of oral hygiene. Therefore, oral hygiene instructions should be given before the initiation of orthodontic treatment and reinforced during every visit. Regular brushing of the teeth is considered the first line of defense in controlling dental plaque. It has been shown that the use of electrical and ultrasonic toothbrushes is superior to manual brushing in controlling bacterial plaque on the buccal surfaces and reducing gingival inflammation (40). However, electric toothbrushes are not recommended for orthodontic patients by the majority of orthodontists.

The use of an interproximal brush in addition to the orthodontic brush is considered necessary. Oral hygiene during orthodontic treatment is key to maintaining a healthy periodontium (41).

Orthodontic treatment is contraindicated for patients with active periodontal disease as the risk for further periodontal breakdown is markedly increased. A complete evaluation of the periodontal status, especially in adult patients, is required, and control of the periodontal status is necessary prior to the initiation of orthodontic treatment (42).

It has been proven that fixed orthodontic appliances can cause temporary changes in the oral microbiome. Main clinical manifestations include an increase in periodontal probing depth, probing hemorrhage, gingival redness, and hyperplasia. However, the extent of permanent negative effects on a patient's gingival tissues caused by fixed orthodontic appliances has not been confirmed. Maintaining patient oral hygiene during orthodontic treatment is reported to be very difficult according to existing evidence from longitudinal clinical studies (8).

Traditional remedies like *Miswak* (prepared from the roots, twigs, and stems of the *Salvadora persica* plant) have been used for over a thousand years in the Middle East to combat oral infections. Its antimicrobial properties may offer a cost-effective alternative to control microbial infections (43).

4.4. Decalcification and caries associated with orthodontic treatment

Caries Etiology

Dental caries arise from acids generated by bacteria in dental plaque. Initially, plaque is a soft, thin biofilm composed of bacteria, mucin, dead epithelial cells, and food debris that develops on the tooth surface within 24 hours post-cleaning. The *Streptococcus mutans* species, a collection of bacteria presents in dental plaque, exhibit significant cariogenic potential, with some strains being more pathogenic than others. Soft plaque mineralizes into calculus (hard plaque or tartar) after approximately 72 hours due to the deposition of calcium, phosphate, and other minerals. This calcified plaque is not readily eliminated with a toothbrush (44).

The progression of dental caries is affected by multiple factors, including microbial, salivary, and environmental elements, as detailed below:

4.4.1. Microbial Factors

The primary culprits are *Streptococcus mutans* and *Lactobacilli*, which metabolize sugars to generate acids that demineralize dental enamel.

4.4.1.1. Role of *Streptococcus mutans*

Streptococcus mutans is a gram-positive coccus that is commonly found in the human buccal cavity and is facultatively anaerobic. The capacity to initiate and sustain microbial growth and to continue acid production at low pH values, the rapid metabolism of sugars to lactic and other organic acids, the ability to attain the critical pH for enamel demineralization more rapidly than other common plaque bacteria, the ability to produce Intracellular Poly-Saccharides (IPSs) as glycogen, which may act as a food store for use when dietary carbohydrates are low, and the significant reduction in the incidence of caries observed in animals immunized with specific *S. mutans* serotypes have been reported (45).

Dental caries are chronic infections that are caused by the normal microbial flora of the mouth. Although there are numerous bacteria species in the buccal cavity, only a specific species of bacteria can cause dental caries and periodontal infection. *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces* are the bacteria that are most frequently linked to dental caries. These are acidogenic and aciduric gram-positive bacteria. *Lactobacillus* is not the cause of caries; however, it is a critical factor in its progression. Although there are numerous bacteria species in the buccal cavity, only a specific species of bacteria can cause dental caries and periodontal infection. *Streptococcus mutans*, *Lactobacillus*, and *Actinomyces* are the bacteria that are most frequently linked to dental caries. These are acidogenic and aciduric gram-positive bacteria. *Lactobacillus* is not the caries initiator; however, it is crucial to the progression of caries (46).

In the mid-1960s, clinical and animal experiment studies identified *Streptococcus mutans* as an important causative agent in dental caries. The main habitat of *Streptococcus mutans* is the human oral cavity, more specifically the dental plaque, where biofilm is formed on all surfaces of the tooth (47).

The cariogenic potential of *Streptococcus mutans* is attributed to three primary characteristics:

1. The enduring colonization of rigid surfaces resulting from the synthesis of a substantial quantity of extracellular glucan polymers derived from sucrose.
2. The capacity to transport and metabolize a substantial quantity of carbohydrates into organic acids. Acidogenicity
3. The capacity to endure environmental stressors, namely low pH levels, is known as aciduricity (48).

Streptococcus mutans may alter the local environment by creating circumstances rich in extracellular polysaccharides and low pH, so facilitating the proliferation of acidogenic and aciduric bacteria, which contributes to the development of dental caries (43).

The concentration of *Streptococcus mutans* varies throughout different areas of the oral cavity, with a notable prevalence on the dorsum of the tongue. The formation of dental plaque encompasses three phases. Salivary molecules first attach to the enamel, forming a covering made up of a complex mixture of components, including

as glycoproteins, acidic proline-rich proteins, bacterial cell debris, exoproducts, mucins, and sialic acid. The last phase entails bacterial engagement with the acquired pellicle via specific cell-to-surface contacts, resulting in biofilm formation by primary colonizers such *Streptococcus sanguis* and *Actinomyces viscosus*. During the third phase, other bacterial species, including *Streptococcus mutans*, adhere to the primary colonizers via cell-to-cell interactions, leading to the development of a biofilm on the tooth, known as dental plaque (49,50).

Streptococcus mutans facilitates the onset and advancement of dental caries by producing extracellular glucans from dietary sucrose. These glucans augment plaque bulk, facilitate the colonization of *Streptococcus mutans*, and modify the diffusion characteristics of the plaque matrix, hence exacerbating its cariogenic potential (51).

Streptococcus mutans secretes glucosyl transferase on its cell wall, enabling the bacteria to synthesize polysaccharides (glucan) from sucrose, which facilitates bacterial aggregation and adherence to tooth enamel, resulting in biofilm formation (51).

A primary virulence component of *Streptococcus mutans* is the synthesis of bacteriocins, known as mutacins, which are peptide antibiotics. *Streptococcus mutans* frequently employs two-component signal transduction systems to modulate bacteriocin gene expression and is also associated with biofilm formation. *Streptococcus mutans* regulates several physiological processes in a cell density-dependent way (52).

4.4.1.2. Role of Lactobacilli

Lactic acid bacteria are a diverse group of bacteria that play an important role in maintaining oral health. These bacteria are primarily known for their probiotic properties, which contribute to a balanced oral microbiota and inhibit the growth of harmful microorganisms (53). However, certain *Lactobacillus* species have similarities such as tooth surface adhesion and acidic production, which can lead to tooth decay (54).

The oral cavity is a complex ecosystem in which lactic acid bacteria interact with other microorganisms. Useful species such as *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, and *Lactobacillus plantarum* contribute to oral health. Conversely, species such as *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus gasseri*, and *Lactobacillus casei* have been found to contribute to tooth decay, particularly at high levels. A study shows that lactic acid bacteria balance in oral microbiomes is essential for the prevention of oral disease. High acid-producing lactic acid bacteria can lower the pH in the oral cavity and promote the growth of cariogenic bacteria such as *Streptococcus mutans* (54).

Conversely, maintaining healthy levels of lactobacilli can support general oral health through good oral hygiene and nutritional practices (55). *Lactobacillus* is primarily considered non-pathogenic, but a few species of lactic acid bacteria are present in tooth decay lesions. *Lactobacillus* species are usually not seen in children without cavities. These bacteria contribute to the progression of cavities but are not the initiators of caries. The main feature of *Lactobacillus* is its ability to produce lactic acid as a by-product of glucose metabolism. *Lactobacillus* is considered the second most frequently occurring cariogenic bacteria in oral flora. The types of lactic acid bacteria that are primarily associated with the pathogenesis of caries are *Lactobacillus gasseri*, *Lactobacillus fermentum*, and *Lactobacillus casei* (56).

The two cariogenic properties of *Lactobacillus* are adhesion and acid production. The cell surface hydrophobicity of *Lactobacillus* is primarily due to the S-layer proteins of its cell wall. Exosugars produced by *Lactobacillus* species are an important factor in the formation of biofilms. These bacteria use two types of metabolic processes to produce lactic and acetic acid. *Lactobacillus* is considered the main organism in the progression of tooth decay due to its high number of caries lesions, acid production, and aciduric properties. It is an excellent acid-resistant bacterium and can survive under pH 4.5. These bacteria bind to dental plaques and begin to demineralize the enamel of the teeth (57).

4.4.1.3. Impact of orthodontic appliances

Orthodontic treatment is a frequent dental procedure that aims to modify occlusion and improve overall oral health. However, the presence of orthodontic devices can significantly impact the periodontal microbiota, leading to changes in the bacterial population that can affect periodontal health. Studies have shown that orthodontic treatments can affect saliva parameters and bacterial values, including *Lactobacillus* species and *Streptococcus mutans* (58). These changes are primarily due to an increase in retention points for plaque accumulation, such as clips and wires, which create an environment favorable for bacterial growth. Correction devices generate retention points for the periodontal microbiota, leading to increased growth of *Streptococcus mutans* and lactic acid bacteria. The extent of bacterial growth depends on factors such as the number of orthodontic brackets, duration of treatment, and oral hygiene (59).

Bacterial adhesion on orthodontic brackets is an important factor in the development of caries and periodontal disease. Studies have shown that different types of brackets can affect the adhesion of bacteria such as *Streptococcus mutans* and *Lactobacilli* (60). This adhesion is influenced by the material and design of the brackets, which can either facilitate or prevent bacterial accumulation. Orthodontic treatment leads to an increase in periodontal pathogenicity, cariogenic bacteria, and corrosion-induced bacteria, raising the risk of gingivitis, periodontitis, tooth caries, and enamel erosion. Studies have shown that the presence of fixed orthodontic devices complicates oral hygiene maintenance, leading to more plaque accumulation and microbiological changes that favor harmful bacteria (61). Effective plaque control and regular dental monitoring are crucial to mitigate these risks and improve health outcomes during orthodontic treatment.

Furthermore, the impact of orthodontic treatment on periodontal health has been of interest for many years. Early studies highlighted the potential for orthodontic devices to disrupt the balance of periodontal microbiota, leading to an increase in periodontal diseases and cariogenic bacteria (62). These microbial shifts can lead to complications such as gingivitis, periodontitis, and tooth caries, highlighting the need for effective plaque control and regular dental monitoring during orthodontic

treatment. Orthodontic treatment can promote the growth of lactic acid bacteria, such as *Lactobacillus* species. Patients receiving orthodontic treatment may experience a significant increase in the population of *Lactobacillus* species (63). Orthodontic devices promote the growth of bacteria in saliva and plaque. The combination of orthodontic treatment and poor oral hygiene can seriously damage periodontal health. Studies have confirmed an increase in *Streptococcus mutans* colonization in orthodontic patients (59,64).

Fixed devices increase bacterial aggregation by creating stagnant areas but also stimulate stable flow, raising the pH and buffering capacity of the oral environment. This phenomenon explains the lack of decalcification in some patients despite high plaque levels (51). Orthodontic devices make oral hygiene difficult by creating stagnant areas that lead to carbohydrate accumulation and retention, promoting demineralization (65).

In summary, orthodontic treatments can promote the growth of harmful bacteria and pose risks to periodontal health. Understanding the relationship between orthodontic devices and bacterial growth is essential to mitigate these risks and develop strategies to ensure successful treatment outcomes.

4.4.2. Salivary Factors

- **Salivary Parameters**

Salivary flow rate, pH levels and capacity for buffering significantly influence mineral loss, progression of demineralization, and potential for remineralization after acidic episodes (67).

- **Impact of Salivary Flow Rate**

Higher salivary flow rates enhance physical cleansing, increase buffering capacity, and accelerate clearance of substrates. Salivary pH, which generally rises with flow rate, helps neutralize plaque acid. For example, unstimulated saliva may exhibit a pH lower than 6, whereas stimulated saliva can attain a pH of 8, facilitating remineralization (51). Saliva helps neutralize acids, provides minerals for

remineralization, and washes away food particles and bacteria, also Saliva's ability to neutralize acids is crucial in preventing caries (67).

4.4.3. Environmental Factors

- **Oral Hygiene Practices**

Inadequate oral hygiene practices such as inadequate brushes and dental flossing can build up plaque on your teeth. Plaque is a sticky film of bacteria that form on teeth and gums. If not removed regularly, bacterial growth, particularly those in which the environments of the minus and lactobacilli are beneficial, is known to contribute to tooth decay. These bacteria metabolize sugar from food and drinks, covering the enamel of the teeth and producing acids that lead to tooth decay. Regular brushes and dental floss help remove plaque and food particles and reduce the risk of tooth decay and periodontal disease. To maintain optimal oral health, we recommend applying flossing every day at least twice a day. Fluoride plays an important role in oral hygiene by enhancing the remineralization process of tooth enamel. If there is fluoride in the mouth, it is attached to the enamel of the teeth and is resistant to acid attacks caused by bacteria metabolism. This process helps to repair the early stages of decay and strengthen the enamel against future decalcification. You can find fluoride in a variety of sources, including drinking water, toothpaste, mouthwash, and specialized treatments that dentists provide. Regular use of products containing fluoride is important for preventing tooth decay and maintaining strong and healthy teeth (68).

- **Dietary Influences**

Frequent carbohydrate consumption results in overlapping acid attacks without adequate recovery periods, leading to a net mineral loss over time (69).

Acidic Foods and Beverages can directly erode enamel and lower the pH in the mouth, promoting demineralization, also.

4.5. Miswak (*Salvadora Persica*)

4.5.1. Synonyms:

"Miswak" (synonyms in various Arabic dialects and countries include "miswaak," "miswak," "miswaki," "meswak," "mswaki," "sewak," "siwak," and "siwaki") is an Arabic word that means tooth-cleaning stick, as was previously mentioned under the introduction and purpose title (18). The dissemination and application of miswaks, a pre-Islamic practice, in various regions of the world were greatly impacted by the spread of Islamic culture (21).

4.5.2. Miswak plant and its characters

Among at least 182 plant species suitable for preparing toothbrushing sticks, miswak harvested from *Salvadora persica* is used most extensively (70). The roots, twigs, and stems of this plant have been used for oral hygiene (71). *Salvadora persica* sticks have been used as toothpicks (19). *S. persica* has a wide geographic distribution, starting from Rajasthan (India), Nepal, and Malaysia in the east, through Pakistan, Iran, Iraq, Saudi Arabia, and Egypt to Mauritania in the west, and from North Africa through Sudan, Ethiopia, and Central Africa to southwestern Africa (1). Taking into account the historical importance of the use of *S. persica* miswak in oral hygiene, the present review aims to remind readers of the significant contributions this practice has made to dentistry, incorporating the latest literature, and to explain the key factors of its impact on oral health, alongside its disadvantages (19).

4.5.3. Chemical composition

Chemical analysis of *S. persica* miswak has indicated the presence of β -sitosterol, M acid acid, chloride, salvaduria, plaster, and organic compounds such as pyrrolidine, pyrrole, and piperidine derivatives (72). It has also been found to contain glycosides such as salvadoside and flavonoids including kaempferol, quercetin, quercetin rutin, and quercetin glucoside (73, 74). The roots and bark of the *S. persica* tree are composed of 27% ash, a high ratio of alkaloids such as salvadrine and trimethylamine, chlorides, and fluorides. Medium concentrations of silica, sulfur, and

vitamin C are present, along with small amounts of tannins, saponins, flavonoids, and sterols (18). Large quantities of sodium and potassium chloride were found, along with other sulfur-containing organic substances like salvadour and salvadrine. *S. persica* miswak contains almost 1.0 mg/g of total fluoride, and it has been shown to release a significant amount of calcium and phosphorus into the water. Repeated chewing of *S. persica* miswak has been found to release fresh juices that may have anti-cariogenic effects (20). However, in another study, a negligible amount (0.07 mg/ml) of fluoride was released when *S. persica* miswak was soaked in water (18).

Benzylisothiocyanate (BITC) was isolated from *S. persica* roots (Farooqi and Srivastava, 1968), and it was shown that BITC is the final product derived from the enzymatic hydrolysis of glucosinolates present in plants (75). BITC is a chemical compound that shares similarities with other genotoxic compounds and can reach or respond to target regions of treated tissues (76). The concentration of BITC was 133.3 µg/mL, and BITC demonstrated viral-type activity against herpes simplex virus II. Furthermore, BITC has a wide range of bacterial activity (77) and inhibits the growth and acid production of *Streptococcus mutans* (78).

Other ingredients in *S. persica* also have beneficial effects on oral health. The sulfur content of *S. persica* root ash was up to 4.73%. Sulfur has a bacterial-killing effect (79), and vitamin C helps in healing and repairing tissues. Silica acts as an abrasive and helps to remove spots on the surface of teeth (18). The astringent effect of tannins helps reduce clinically detectable gingivitis. Tannins were found to inhibit the effect of glucosyltransferases, thereby reducing plaque and gingivitis (80). Tannins can form layers on enamel that protect against tooth decay (18). Salvadrine, an alkaloid found in *S. persica* miswak, has a bacterial effect and can stimulate the gums. The slightly bitter taste of *S. persica* essential oil stimulates the flow of saliva, acting as a buffer. High chloride concentrations inhibit the formation of calculus and help remove spots on the surface of teeth (25,80).

4.5.4. Effects of miswak on oral health

4.5.4.1. Antimicrobial characteristics

An in vitro analysis revealed that the water extract of *S. persica* miswak exhibited a suppressive influence on the proliferation of *Candida albicans*, likely due to its elevated sulfate concentration (81).

Derivatives of *Salvadora persica* miswak were examined using three distinct laboratory methods, with significant antimicrobial properties demonstrated against *Streptococcus* sp. and *Staphylococcus aureus* (18).

Additionally, it was found that *Enterococcus faecalis* was the most responsive microorganism affected by *S. persica* miswak. No significant differences were noted in the antimicrobial effects between freshly cut and 1-month-old miswak. A comparison between the alcoholic and aqueous extracts of *S. persica* miswak revealed that the alcoholic extract exhibited stronger antimicrobial activity than the aqueous extract (82, 83).

Extracts from *S. persica* miswak possessed antimicrobial effects on *Streptococcus mutans* and *E. faecalis*. It was proposed that this effect might stem from interactions with bacteria that hinder their adhesion to tooth surfaces (84,85). Significant variances in the antimicrobial efficiencies of the pulp and bark extracts from *S. persica* miswak were also observed (86). Research has identified various anionic constituents of *S. persica* miswak recognized for their antimicrobial effects (37). These components were theorized to exert robust promoter effects on salivary peroxidase thiocyanate and hydrogen peroxide antimicrobial mechanisms (87).

Additionally, a study conducted among Sudanese adults examined the salivary levels of 25 oral pathogens, suggesting that *S. persica* miswak might have a selective suppressive effect on specific bacterial levels. Miswak users had significantly lower quantities of cariogenic bacteria, except for *Streptococcus mutans*, in their saliva, while toothbrush users demonstrated lower salivary concentrations of periodontal pathogens. The periodontal pathogen *Aggregatibacter actinomycetemcomitans* and

other bacterial species were found in significantly greater numbers in the saliva of miswak users compared to toothbrush users (37). Furthermore, another study indicated that miswak users exhibited notably higher plaque levels of *Staphylococcus intermedius*, *A. actinomycetemcomitans*, *Veillonella parvula*, *Actinomyces israelii*, and *Capnocytophaga gingivalis*, along with significantly lower levels of *Selenomonas sputigena*, *Streptococcus salivarius*, *Streptococcus oralis*, and *Actinomyces naeslundii* than toothbrush users (88).

In a single-blind, randomized, crossover examination involving 15 Saudi Arabian participants, it was demonstrated that the impact of chewing miswak on the subgingival plaque microbiota levels was comparable to that of standard tooth brushing without toothpaste, with *A. actinomycetemcomitans* levels significantly reduced more with miswak than with tooth brushing (89). Immediate antimicrobial effects of *S. persica* miswak and its extract on *Streptococcus mutans* and *Lactobacillus* were investigated, showing a significant reduction in *Streptococcus mutans* counts but no effect on *Lactobacillus* counts among miswak users (90). Based on their experimental findings, it was recommended to incorporate *S. persica* miswak extract into mouthwashes and toothpaste due to its notable antimicrobial effects (91). Their research included three in vitro studies examining the impact of *S. persica* miswak extract on specific bacteria, comparing the paraclinical effects of Iranian toothpaste containing *S. persica* miswak extract against a placebo toothpaste on dental plaque, and contrasting the antibacterial efficacy of Iranian toothpaste with both Swiss toothpaste on dental plaque and penicillin. Results from all three studies highlighted the beneficial paraclinical effects of *S. persica* miswak extract on dental plaque. An assessment of complete (unextracted) *S. persica* miswak segments embedded in agar or suspended above the agar resulted in robust antibacterial effects against oral microorganisms associated with periodontitis and dental caries (23).

Fluoride, a component of *S. persica* miswak, demonstrated a potential interaction with bacterial glycolytic enzymes and their acids. Additionally, prior research suggested that the BITC component of *S. persica* miswak acted as a bacterial growth suppressor. Both aqueous and methanol extracts of *S. persica* miswak were evaluated for their antimicrobial activities against seven isolated oral pathogens (*S.*

aureus, *Streptococcus mutans*, *Streptococcus pyogenes*, *E. faecalis*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, and *Candida albicans*) using two methods. Both antimicrobial assays indicated that the aqueous extract inhibited all isolated microorganisms and was more effective than the methanol extract, which was resisted by *L. acidophilus* and *P. aeruginosa*. The strongest antibacterial effect was displayed by the aqueous extract against *E. faecalis*. Turbidity tests showed that both extracts had comparable antifungal activity against *C. albicans* (92). The derivatives of *S. persica* miswak are reported to exhibit pronounced antimicrobial activity, with these diverse components extractable through various chemical methods (93).

4.5.4.2. Anticariogenic features

Many epidemiological studies have reported that *S. persica* miswak has a strong anti-caries effect. In a study conducted as part of the Sudan Dental Health Survey, it was found that miswak users had a lower prevalence of dental caries (94). Subsequent studies reported similar findings, with schoolchildren using miswak showing lower rates of tooth decay (95). It was also reported that chewing sticks are more effective at reducing tooth decay than traditional toothbrushes (96). Despite the carbohydrate-rich diet traditionally consumed in Ghana, the incidence of tooth decay and other dental diseases among Ghanaian chew stick users was low. A cross-sectional study among adults in West Africa also reported a decline in caries and plaque in miswak users compared to non-users (97). The sharp taste and chewing effect of miswak can increase saliva production, thereby enhancing the mouth's buffering capacity (18). In in-vivo studies, it was found that rinsing with *S. persica* miswak extract stimulates parotid glandular secretion and increases the pH value of plaque. This effect can potentially prevent tooth decay by reversing the acid challenge of cariogenic bacteria (23). In Zanzibar, the prevalence of tooth decay was lower in rural areas, and the use of miswak was more common than in urban areas (98). Comparative studies have shown that both aqueous extracts of miswak and walnut extracts (from *Juglans regia*) can significantly inhibit the growth of cariogenic bacteria (99). The large amount of fluoride available in miswak can contribute to this effect (72). However, previous studies have shown that this potential fluoride contribution is

questionable because the total fluoride solution is negligible when *S. persica* is soaked in water (18).

4.5.4.3. Effects on Dental Plaque, Gingival Health, and Periodontal Status

The regular use of *S. persica* miswak has been linked to sustained plaque reduction and improved oral hygiene over the years. Its herbal composition and simplicity of use make it an appropriate long-term oral hygiene tool (100). Many reviews have revealed that *S. persica* miswak effectively reduces gingivitis and dental plaque. It has been shown that *S. persica* miswak removes plaque more effectively than toothbrushing in studies involving Ethiopian schoolchildren and Saudi Arabian dental students. The study involving schoolchildren required the provision of instructions and supervision, as most children were unfamiliar with the proper technique of miswak use (34,96).

The use of *S. persica* miswak in preventive dental programs has been recommended because it is economical and familiar to older individuals (96). A significant plaque reduction of 75% was reported after eight days of using *S. persica* miswak (101). A study conducted among groups of students in Kenya indicated that no additional approach was needed to eliminate dental plaque in the group using toothpaste in combination with chewing sticks (102). Although *S. persica* miswak can efficiently remove dental plaque, an association between excessive miswak use and gingival recession was confirmed in Saudi schoolchildren (103).

Many cases of gingival recession among miswak users have been reported, which may be due to mechanical trauma (104). However, no significant difference was found in gingival indices or bleeding between miswak and toothbrush users. Gingival indices were found to be significantly lower following the use of *S. persica* miswak compared to using a conventional toothbrush without toothpaste (105).

Several toothpastes containing *S. persica* miswak extract are commercially available, and one such toothpaste was found to be significantly more effective in removing dental plaque compared with Oral-B toothpaste (18,106).

The combined effect of mechanical cleaning and enhanced salivation achieved with the proper use of *S. persica* miswak was found to be more efficient than toothbrushes in removing dental plaque (1). In a randomized crossover study among 15 Saudi Arabian male volunteers, it was found that miswak use significantly reduced plaque and gingival indices and was more effective than toothbrushing when preceded by professional practice concerning its accurate use (107). Rinsing with a slurry of toothpaste containing *S. persica* miswak has been shown to reduce gingival inflammation and bleeding on probing. In contrast, chlorhexidine was found to reduce plaque more effectively than *S. persica* miswak, and the anti-plaque effects of both products suggested no definite advantage in using *S. persica* miswak over chlorhexidine.

In a similar study investigating a commercial herbal mouthwash containing *S. persica* miswak extract, large reductions in gingival bleeding were found in both test and placebo groups. However, a significant reduction in the load of cariogenic bacteria was found only in the test group (108).

The efficacy of chewing sticks has been challenged in several studies. More plaque formation and gingival bleeding were reported in individuals using chewing sticks than in toothbrush users (97). Habitual users of miswak were found to have a significantly higher incidence of gingivitis than toothbrush users (97,109).

Miswak sticks were indicated to be less effective than toothbrushes in cleaning interproximal dental regions and lingual surfaces (105). Low levels of enamel loss in adults were found in countries where miswak is widely used (19).

Epidemiological research has suggested that miswak use has beneficial effects on the prevalence of periodontal diseases and caries (110,111). Moreover, low periodontal treatment needs were reported among Saudi adults who used miswak (110,112). An epidemiological study conducted among certain nomadic groups in the Kaisut desert region of Kenya confirmed that dental caries and advanced periodontal disease were rare among miswak users under the age of 50 years. However, a retrospective study conducted in Saudi Arabia showed conflicting results, where miswak users had deeper periodontal pockets and a higher incidence of periodontal

diseases than non-users (113). In a study conducted at a medical campus in Sudan, it was reported that the periodontal status of routine miswak users was similar to or slightly better than that of toothbrush users (114).

4.5.4.4. Comparative Effectiveness of Miswak and Traditional Toothbrushes

Studies comparing miswak with traditional toothbrushes have shown that miswak is equal to or more effective in reducing plaque and improving gum health. In some studies, miswak users had lower plaque values and improved periodontal health compared to traditional toothbrush users. The physical effects of using miswak as a caustic or toothbrush help to remove plaque from the tooth surface. The fibrous nature of the miswak stick resembles the bristles of a toothbrush and effectively cleans teeth and gums (90,107,115).

Furthermore, miswak use was associated with reduced gingivitis and bleeding. This is particularly advantageous for orthodontic patients at high risk of gingivitis, as oral hygiene is maintained, and other orthodontic devices are preserved. The natural compounds found in miswaks, such as silica, tannins, and essential oils, contribute to anti-inflammatory and strengthening properties, promoting healthier gums and overall oral health (108,116).

Furthermore, the convenience and cultural acceptance of miswak in many regions can improve adherence to oral hygiene practices and lead to better long-term outcomes (20,80).

4.6. Oral Hygiene

Oral health is an important part of overall health, especially for patients without teeth who depend on dentures for daily activities. Denture-induced stomatitis (DIS) is a common issue for these patients, marked by swelling and pain in the mucosal tissues due to inadequate oral care and plaque buildup on dentures. Traditional oral care

methods, although effective, may not fully manage DIS. As a result, interest is growing in natural options like Miswak (*Salvadora persica*), which is recognized for its antimicrobial and anti-inflammatory effects. Miswak has been used historically for oral hygiene in many cultures and has shown possible benefits in reducing plaque and enhancing gum health. This study intends to investigate how effective Miswak is as an additional oral hygiene tool for denture users, concentrating on its capacity to enhance oral care and relieve symptoms related to denture-induced stomatitis (117,118).

Recent research has shown that miswak effectively improves oral health over relatively short timeframes. A study lasting 3 months examined the mechanical removal of plaque and benefits to gum health from miswak compared to standard tooth brushing. The outcomes indicated that miswak was just as effective, if not more so, in decreasing plaque and enhancing gum health. The fibrous structure of the miswak stick, functioning like toothbrush bristles, greatly enhanced its ability to clean mechanically, efficiently removing plaque from tooth surfaces and supporting healthier gums (119).

Additionally, the effects of miswak on gum health were assessed through multiple randomized controlled trials lasting 4 to 6 weeks. These trials consistently indicated that using miswak resulted in less gum inflammation and bleeding, which is especially helpful for orthodontic patients who struggle to maintain oral hygiene around braces and other devices. Similarly, the immediate effects of miswak on oral cleanliness were studied over 2 months, observing significant improvements in oral hygiene and a decrease in harmful bacteria. These studies highlight the promise of miswak as a useful tool for supporting oral health, particularly in groups facing unique oral hygiene challenges (120,121).

The effectiveness of *S. persica* miswak mainly comes from its mechanical cleaning ability (122).

Notably lower plaque levels were found after proper miswak use as an oral hygiene aid compared to using regular toothbrushes (105).

Miswak is typically used for a longer period than a toothbrush, and it is easier to reach the buccal/labial surfaces of the teeth than the lingual and proximal surfaces. Despite the extended use of miswak, no significant difference in buccal/labial plaque levels was noted between miswak and toothbrush users. In many developing nations, chewing sticks are often the only devices used for oral cleaning (123).

Oral hygiene upkeep through the consistent elimination of dental plaque is a crucial element in preventing dental cavities and gum disease. Several reasons for the cleaning effectiveness of *S. persica* miswak have been suggested, including (a) the physical impact of its fibers, (b) the release of beneficial substances from the chewing stick during use, or (c) a mix of both. However, the absence of research indicating the time, length, and frequency of *S. persica* miswak use limits useful evaluations of its mechanical cleaning effects on oral health (124).

In a 2005 survey, most Jordanians thought that using a toothbrush and miswak was the best way to achieve good oral health (125). *Salvadora persica* miswak is viewed as an affordable and easily accessible oral hygiene tool that most people can use (126).

4.7. Disadvantages of Chewing Sticks

S. persica miswak is considered an essential tool for maintaining oral hygiene, but certain drawbacks are related to its use. The bristles are aligned along the long axis of the rod, whereas those on the toothbrush are placed perpendicular to the handle. Therefore, reaching the tongue with miswak can be difficult. Another drawback is related to the habitual use of miswak over a long period, which may lead to some issues (127). It has been reported that miswak users may overscrub the front teeth while neglecting the posterior teeth (128). Other studies suggest that the use of miswak may be one of the potential pathogenic factors contributing to gingival recession (72).

These drawbacks can be overcome by providing proper methods and clear instructions from the dentist regarding the duration of miswak use. The potential cytotoxicity of *S. persica* plants and their diffusive components on oral tissues was

evaluated using tissue culture overlay methods. The results indicated that *S. persica* miswak had no cytotoxic effect when freshly slit, but after 24 hours, the same plant contained harmful ingredients (129). Studies on the effects of direct administration of high doses of *S. persica* extracts on mice resulted in several minor side effects on the reproductive system and fertility in both men and women (130). However, previous studies indicated that neither aqueous nor ethanolic *S. persica* extracts were toxic to mice at doses of up to 1200 mg/kg (131).

4.8. Investigating The Effects of Miswak On Orthodontic Patients

- **Orthodontic Patients Face Unique Oral Hygiene Challenges**

Orthodontic patients, particularly those with fixed appliances like braces, face specific difficulties in maintaining oral hygiene. The brackets, wires, and bands create hard-to-reach areas that trap food and plaque, increasing the risk of dental issues such as plaque buildup, gum inflammation, and cavities. Given these challenges, effective oral care strategies are essential for orthodontic patients.

- **Potential Benefits of Miswak's Antibacterial Properties**

Miswak has long been recognized for its natural antibacterial, anti-inflammatory, and antimicrobial properties. These characteristics suggest that miswak could be beneficial in controlling plaque accumulation and supporting gum health in orthodontic patients. However, there is a notable lack of research specifically investigating its effects on this population. This gap presents a valuable opportunity to explore whether miswak can provide an effective, natural solution for improving oral hygiene during orthodontic treatment.

- **Oral Health and Gum Inflammation in Orthodontic Treatment**

Orthodontic treatment often results in gingival inflammation and discomfort, particularly around braces, due to the difficulty of cleaning teeth thoroughly. Miswak's anti-inflammatory properties may offer a natural alternative to conventional oral care products by reducing gum swelling and bleeding. The absence of studies on miswak's impact on gum health in orthodontic patients leaves an important research question unanswered.

- **A Growing Demand for Natural Oral Care Solutions**

With increasing consumer demand for natural products, orthodontic patients may prefer using herbal alternatives to chemical-based oral care products. Miswak, a natural and well-established oral hygiene tool, offers the potential to meet this demand. Exploring its effects on orthodontic patients could offer a new, natural option for improving their oral health during treatment.

In recent years, natural oral care solutions have gained significant attention due to their health benefits and cultural acceptance. Among these, *Salvadora persica* miswak has been widely studied for its antimicrobial, anti-inflammatory, and astringent properties, all of which contribute to improved oral hygiene and periodontal health. While an increasing body of research supports the effectiveness of natural products like miswak, a notable gap remains in studies specifically evaluating their impact on orthodontic patients.

Individuals undergoing orthodontic treatment face unique oral hygiene challenges due to braces and other appliances, which complicate plaque removal and increase the risk of gingivitis. Although numerous studies have investigated the benefits of natural oral care solutions in general populations, only a limited number have examined their effectiveness in orthodontic patients. Recent systematic reviews suggest that natural products may help reduce inflammation, bacterial levels, and oxidative stress in the oral cavity. For example, studies have reported significant

improvements in periodontal health following the use of miswak-infused toothpaste and other natural oral care products (119,120).

Given the mechanical difficulties and increased susceptibility to gingivitis in orthodontic patients, further targeted research is needed to explore the long-term effects of natural oral hygiene solutions in this group. Addressing this research gap could provide valuable insights and potentially lead to the development of specialized natural oral care products designed to meet the unique needs of orthodontic patients.

4.9. Post-Treatment Care After Fixed Appliance Removal

While the ideal approach is to prevent demineralized white spot lesions (WSLs), these lesions may still develop despite preventive efforts. Their management should begin with the most conservative treatments and escalate to more intensive interventions only when necessary (132).

Conservative strategies focus on remineralizing lesions, with the choice of treatment depending on lesion status and depth. Various commercial products claim to support the remineralization process (133).

One of the most common remineralization agents is topical fluoride, available in the form of toothpaste, gels, mouth rinses, or varnishes. Fluoride enhances saliva-mediated remineralization post-debonding by promoting the formation of fluorapatite, which strengthens enamel (134). However, high concentrations of fluoride may cause surface hypermineralization, potentially leading to staining, whereas low-concentration fluoride formulations are generally recommended to promote uniform remineralization (135).

Research suggests that titanium tetrafluoride (TiF₄) applications can effectively prevent demineralization around orthodontic brackets (136,137). Additionally, the regular use of sodium fluoride (NaF) rinses and high-concentration fluoride varnishes has been associated with a significant reduction in lesion incidence (72,138).

Although fluoride is highly effective in preventing caries and promoting enamel remineralization, excessive exposure can result in dental and skeletal fluorosis, as well as other systemic health concerns (139,140).

Table 4.1. Comparing Fluoride Toothpaste and Miswak

Feature	Fluoride Toothpaste	Miswak (<i>Salvadora persica</i>)
Caries Prevention	Effective in preventing caries by remineralizing enamel	Provides antimicrobial effects that help prevent caries naturally
Plaque Reduction	Controls plaque formation with chemical agents	Contains natural antimicrobial and abrasive compounds
Gingival Health	No direct anti-inflammatory effect	Contains tannins and flavonoids that reduce gingival inflammation
Systemic Risks	Fluoride toxicity can cause fluorosis, thyroid issues, and neurodevelopmental concerns	No known systemic toxicity when used properly
Salivary Stimulation	No direct effect on saliva production	Naturally increases salivary flow, aiding in oral defense mechanisms
Suitability for Orthodontic Patients	Effective but requires additional cleaning tools due to appliance-related plaque retention	Offers a flexible, mechanical cleaning method that can reach interdental areas

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), derived from milk, is essential in stabilizing elevated concentrations of calcium and phosphate ions, which aid in the remineralization of enamel lesions. For lesions that have progressed beyond the stage of effective remineralization, resin infiltration is a viable technique that halts further lesion advancement by penetrating and sealing the affected enamel (51).

One of the primary causes of enamel demineralization during orthodontic treatment is biofilm accumulation around braces. Proper oral hygiene maintenance, including the use of manual or electric toothbrushes along with fluoride-based pastes and rinses, is essential in minimizing the risk of lesions (138). Notably, studies indicate

that white spot lesions tend to diminish significantly within 12 weeks following the removal of fixed appliances (71).

While fluoride-based products have been widely used for remineralization, the literature highlights both their benefits and potential risks. In contrast, miswak has gained attention as a natural alternative due to its antimicrobial and anti-inflammatory properties. However, additional research is required to fully evaluate its long-term effectiveness, particularly for orthodontic patients who face unique oral hygiene challenges.



5. MATERIALS AND METHODS

5.1. Materials

The research, which was conducted on 30 patients for two months, required the use of these materials:

5.1.1. Utensils for cleaning of teeth

- **Figure 5.1.** Miswak (*Salvadora Persica*) sticks



- **Figure 5.2.** Standard soft toothbrush



- Natural toothbrush with miswak tufts (fig. 5.3.) Although not used in this study, it is shown as an example.

Figure 5.3. Natural Toothbrush with Miswak



- Toothpaste – Colgate*

Ingredients:

Glycerin, Aqua, Hydrated Silica, Sodium Fluoride (1450 ppm), Sodium Methyl Cocoyl Taurate, Arginine, Aroma, Cellulose Gum, Zinc Oxide, Benzyl Alcohol, Poloxamer 407, Zinc Citrate, Cocamidopropyl Betaine, Xanthan Gum, Sodium Saccharin, Phosphoric Acid, Sucralose, Sodium Lauryl Sulfate (SLS).

- Dental Floss (Oral B)

5.1.2. Materials and equipment for bacterial counting

The following materials and equipment were used in the microbiology laboratory for bacterial counting:

- ☐ Gram Staining Kit (Reagents) (Fig5.6.)
- ☐ Sheep blood agar
- ☐ Man-Rogosa-Sharpe (MRS) agar
- ☐ Anaerobic jar
- ☐ Gas-pak
- ☐ Glass slide
- ☐ Inoculating loop
- ☐ Bunsen burner
- ☐ Staining rack
- ☐ Wash bottle (Tap water)
- ☐ A compound microscope with 10,40,100X objective lens
- ☐ Grinding machine
- ☐ Vortex Mixer

Figure 5.4. Bunsen burner



Figure 5.5. Anaerobic jar



Figure 5.6. Gram Staining kit



Figure 5.7. Gas-pak



Figure 5.8. Glass slides



5.2. Method

5.2.1. Composition of the sample

The study involved 30 participants divided into two groups, MG and TG, with a 2:1 allocation ratio. The MG group, consisting of 20 participants, used miswak (*Salvadora persica* sticks) for oral health management, while the TG group, comprising of 10 participants, used commercially available toothpaste.

The preferred age range for participants was 12–25 years, and only non-smokers were selected.

Data on the participants' medical and dental history, age, gender, height, weight, BMI, blood group, toothpaste use frequency, and dental floss use were collected.

- The study primarily included **systemically healthy individuals**, while participants who had **regularly used systemic antibiotics, anti-inflammatory, or antioxidant drugs** in the **last three months** were **excluded from both groups**.

Exclusion Criteria:

- Individuals who **underwent surgical periodontal treatment** within the past **12 months**.
- Patients currently taking **medications that impact gingival health**, including **calcium channel blockers, phenytoin, cyclosporine, or hormone replacement therapy**.
- Individuals are diagnosed with **oral cavity neoplastic lesions, diabetes, or rheumatoid arthritis**.
- **Pregnant or lactating women and smokers**.

Participant selection was based on the **2017 AAP/EFP classification** of **periodontal and peri-implant diseases**. The **periodontal status** of each individual was assessed with **meticulous precision** to ensure eligibility.

Inclusion Criteria:

- Individuals with **well-maintained oral hygiene**, no **history or symptoms of periodontal disease**.
- **Probing pocket depth (PPD) $\leq 3\text{mm}$.**
- **Bleeding on probing (BOP) $\leq 10\%$.**

Exclusion Criteria:

- Participants with **interdental or buccal radiographic bone loss of $\geq 2\text{mm}$** involving **non-adjacent teeth**.
- **Radiographically evident bone loss up to 15%** with a **PPD of $\geq 3\text{mm}$** affecting at least **two teeth** were classified as having **periodontal disease** and were **excluded**.

5.2.2 Ingredients in Miswak-Based Toothpaste

- **Active Ingredient**
 - **Miswak Extract (*Salvadora persica*):** The key active ingredient that provides the toothpaste with its natural antibacterial, anti-inflammatory, and cleansing properties. Miswak has been used for centuries for oral hygiene, making it a natural and trusted ingredient in dental care.
- **Humectants**
 - **Glycerin (or Sorbitol):** These ingredients maintain moisture within the toothpaste, preventing it from drying out and ensuring that it retains its paste-like consistency during use.
- **Surfactants**
 - **Sodium Lauryl Sulfate (SLS) (or Cocamidopropyl Betaine):** These compounds help the toothpaste to foam when brushed, assisting in spreading the paste evenly throughout the mouth for better cleaning and a more effective brushing experience.

- **Binders/Thickeners**
 - **Xanthan Gum (or Carbomer):** These ingredients provide the desired consistency for the paste, helping to hold the various ingredients together and ensuring they stay well-mixed.
- **Flavors**
 - **Peppermint oil** (or other **natural flavors**) enhance the taste of the toothpaste and provide a refreshing sensation during brushing.
- **Preservatives**
 - **Sodium benzoate** (or **potassium sorbate**) acts as preservatives, helping to extend shelf life and prevent microbial growth, ensuring the toothpaste remains safe for long-term use.
- **Solvents**
 - **Water:** Water is a primary solvent in toothpaste formulations, helping to dissolve and mix the ingredients for smooth consistency and effective application.

5.2.3. Challenges in Formulation and Production of Miswak Toothpaste

During the initial stages of this research, attempts were made to formulate a miswak toothpaste using appropriate base materials. However, several challenges arose:

- **Inability to Source Base Materials:**

The required base materials for the toothpaste formulation were not available from local suppliers and sourcing them proved difficult due to limited access or specialized requirements.

- **Manufacturing and Regulatory Constraints:**

Local manufacturing facilities could not accommodate the production of the toothpaste, as the formulation would require government approval for production,

particularly regarding safety and regulatory standards. This approval process could not be completed within the timeframe of this research.

- **Use of Commercial Miswak Toothpaste as a Base:**

Due to these limitations, a commercially available miswak toothpaste, which did not include ingredients specifically targeting bacterial count, was selected as the base for the formulation. This toothpaste already contained the necessary components for consistency and use but lacked the addition of active antibacterial agents. To enhance the miswak properties, approximately 5% of powdered miswak was incorporated into the product. However, there isn't a specific "high dose" of miswak usage documented in scientific literature. Most studies focus on its antibacterial properties and effectiveness in oral hygiene rather than dosage

- **Drying Techniques for Miswak Sticks**

The quality of miswak sticks is largely determined by the drying process before they are powdered and formulated into toothpaste. Research has explored how different drying methods affect the preservation of active compounds in herbal materials. Air-drying, though a slower process, has been shown to maintain the chemical integrity of plant compounds, minimizing the loss of essential oils and bioactive components. This method is particularly preferred for delicate herbs, where retaining the full spectrum of natural constituents is essential.

In contrast, oven-drying, when conducted at low temperatures (40-60°C), can expedite the process while still retaining the efficacy of the plant material. Low-heat oven-drying did not significantly alter the quality of the herbs, making it a practical choice for large-scale production of herbal powders. This method is often preferred for industrial applications, as it ensures efficient moisture removal while maintaining the stability of the plant's bioactive properties.

For the purpose of this study, miswak sticks were dried using a controlled oven-drying process to ensure the effective removal of moisture while preserving the active ingredients necessary for the formulation of miswak toothpaste.

- Although *Salvadora persica* (Miswak) is available in various forms in some markets, it is often not fresh. Therefore, we prepared our own miswak toothpaste with the intention of obtaining accurate results from the extensive number of tests planned.

5.2.4 Saliva Collection Protocol and Study Timeline

Saliva samples were collected from two designated groups following a standardized protocol:

1. Patient Consent and Preparation
 - a. Participants were informed about the study protocol, and written consent was obtained before sample collection.
 - b. Prior to saliva collection, participants performed oral hygiene and rinsed thoroughly with distilled water to ensure sample consistency.
2. Saliva Collection Method
 - a. Patients were instructed to sit comfortably and spit into designated tubes five times per minute for a total of 10 minutes, resulting in approximately 50 spits per session.
 - b. Collected samples were transferred to a nutrient growth medium in the microbiology laboratory for further analysis.

Study Design and Follow-Up Protocol

To ensure patient compliance and account for budget constraints, the study was restricted to two time points to minimize the risk of dropouts over an extended monitoring period.

- One month before follow-up, all participants underwent dental scaling and root planing as part of standard periodontal treatment.
- Each subject received a standard toothbrush, dental floss, and a plain Colgate-brand toothpaste without anti-plaque agents.

- The Bass toothbrushing technique was introduced, and patients were instructed to:
 - Brush three times daily for at least two minutes.
 - Floss once per day, preferably before bedtime.

Study Time Points

1. First Appointment – Scheduled 30 days post-routine periodontal treatment to collect the initial saliva sample.
2. Final Appointment – Held 60 ± 2 days after the first visit to collect the final saliva sample.

During both visits, periodontal and gingival indices were measured to assess changes in oral health status over the study period.

The microbiological analysis

Before conducting the desired analysis, test tubes were sterilized using an autoclave machine (fig. 5.9.) at 121°C for 15 minutes.

Autoclaving is considered the gold standard for sterilizing laboratory equipment, particularly liquid-handling instruments, as it effectively eradicates bacteria, viruses, fungi, and spores. This method operates by applying high-pressure steam, which elevates the boiling point of water, ensuring thorough microbial elimination.

When the autoclave door is securely locked, the sealed chamber is evacuated of air by a vacuum pump, which replaces it with steam. High pressure is then applied to the steam, achieving sterilization at the desired temperature for a specific time duration. Once the cycle is complete, the steam is safely exhausted, and sterilized equipment is carefully removed from the chamber.

After the sterilization cycle, test tubes were carefully removed using insulated gloves to avoid contamination or injury from residual heat.

Figure 5.9. Steam sterilization of liquid and culture media.



Prepared growth media plates were inoculated with two different dilutions before being placed in a CO₂ incubator (fig 5.13). The incubator maintained optimal temperature, humidity, and environmental conditions necessary for microbial growth over a 72-hour period. This controlled incubation process ensured proper cultivation while safeguarding the samples from contamination by unwanted bacteria or microorganisms.

The date, dilution rate, type of medium, and the name of the research participants were written on the top of the petri dish and the tubes in a simultaneous form. This prevented cross-sampling and ensured that each result belonged to the same participant.

Saliva samples were placed into sterile test tubes (fig 5.11) and a separated 100 µl amount was transferred into another test tube containing 1 mL sterile saline using a microbiology pipette (5.10.). When the button at the top is pressed with the thumb, a vacuum is created inside the device, through which the liquid is sucked up into the so-called pipette tip. Tips are one-time uses per sample and dilution. On the pipette, you can adjust the exact volume (µl, one-thousandth of milliliters) of liquid you want to

suck up. Once in the pipette tip, the liquid with microbes, for example, can be easily displaced.

Figure 5.10. A pipette is used to very accurately displace a volume of liquid



Figure 5.11. Stack Rack Test Tube Racks



The tubes were aligned based on the dilution rate. Near the Bunsen burner (Fig5.4), each tube was mixed using a vortex mixer (fig 5.12) at low speed for 10 seconds after every transfer. This step was performed to suspend cell or tissue samples for tissue analysis and cell culture. Dilutions ranging from 10^2 to 10^5 were prepared from the centrifuged suspension, and 100 μL of each dilution was used for bacterial culture preparation.

Figure 5.12. High-Speed Test Tube Vortex Mixer with Touch Activation



5.2.5. Growth media

The following growth media were used in our research work

1. **Blood agar** (Trypticase Soy Agar II with 5% Sheep Blood, BD BBL) was selected as a general-purpose growth medium and also as a control agent.
2. Initially, **TYCSB agar** (HiMedia Laboratories) was intended as a selective growth medium for *Streptococcus mutans*. However, due to market unavailability, it was not used in this study.
3. **Man-Rogosa-Sharpe (MRS) agar** (Merck) growth medium used for *Lactobacillus spp.* incubated for 24-72 hours at 37°C in an atmosphere of 10% CO₂.

* A summary description of growth media properties is provided in the appendix.

After the prepared Petri dishes were checked, participant saliva samples were evenly distributed over the surface of the Petri dishes using inoculating Loops (fig 5.14.). An inoculating loop, also known as a smear loop or micro-streaker, is a tool used by biologists to collect inoculum through the phenomenon of surface tension. The

loop is employed to streak agar in a petri dish or plate for microbial cultivation and subsequent growth. Its consistent size ensures accurate and repeatable transfers.

Figure 5.13. A microbial incubator



Figure 5.14. Inoculating Loops



Figure 5.15. A compound microscope

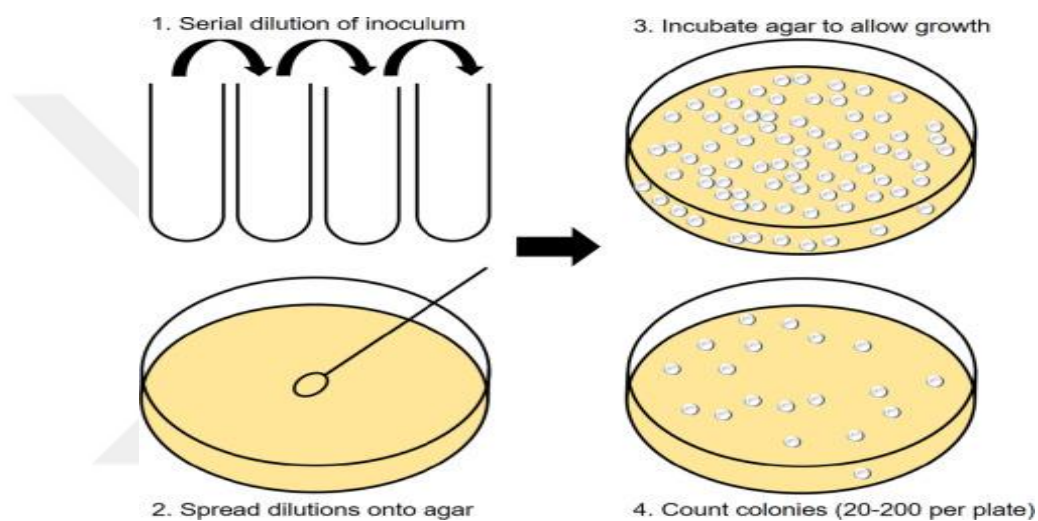


After cultivating each sample, the Petri dish was inverted so that the media was on top. This prevents steam condensation on the lid, which could mix with the media and compromise its integrity. The Petri dishes were incubated at 37°C in a 10% CO₂ atmosphere for 24 to 72 hours to support microbial growth.

The samples were taken out of the incubator in batches and carefully examined to ensure that they were free of fungus or mold since their characteristics were easily identifiable. Then two colonies of different shapes and sizes were selected, and microscope slides were prepared with the sample details recorded.

A drop of normal saline was placed on one end of each slide using a blue stick held perpendicular to the slide, bacterial cells were gently extracted from the dish and mixed with the saline. The slide was then quickly passed over the Bunsen burner 3 times and placed on the table to dry. To fix the dry smear, it was passed through the flame quickly 3 to 4 times, ensuring the smear side faced upward during the process.

Figure 5.16. Identification of growth media



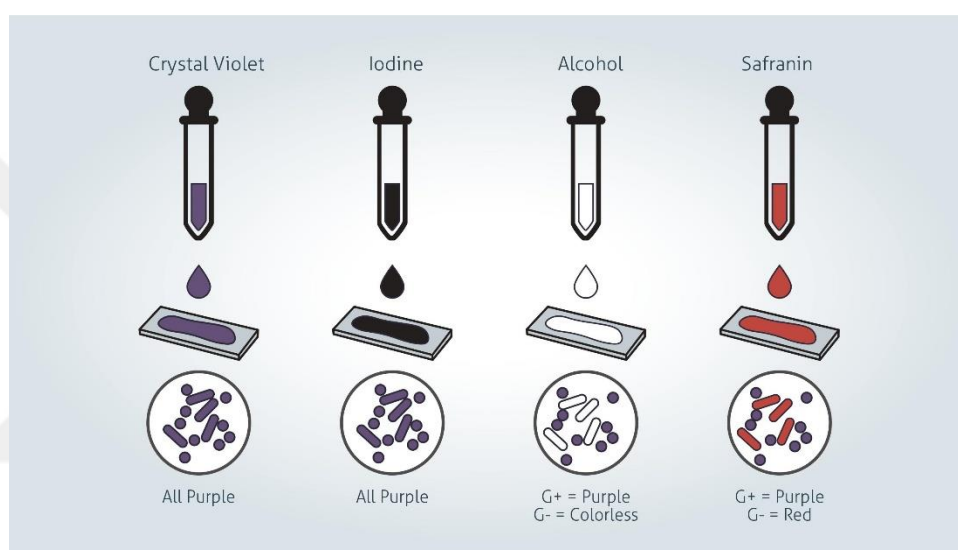
5.2.6. Gram staining procedure

The Gram staining technique (fig. 5.17g) was carried out following these sequential steps:

1. The specimen slide was coated with crystal violet stain and left undisturbed for one minute.
2. The stain was gently removed by rinsing with running tap water.
3. Gram's iodine solution was applied to the slide, ensuring full coverage, and left for one minute.
4. The iodine solution was then carefully rinsed off using a gentle stream of tap water.

5. A decolorizing agent (acid-alcohol) was added to the slide and allowed to act for 20–30 seconds.
6. The slide was immediately rinsed with tap water in a controlled manner and fully drained.
7. A secondary stain, safranin, was applied for 30 seconds to one minute.
8. The slide was washed using a gentle stream of tap water until the runoff appeared clear, followed by drying with absorbent paper.

Figure 5.17. Gram staining procedure



A Gram test was conducted to identify bacterial phenotype. This staining method distinguishes bacteria according to their cell wall composition. Gram-positive bacteria, which possess a thick peptidoglycan layer, appear blue to purple, while Gram-negative bacteria, characterized by a thinner peptidoglycan layer, take on a red to pink coloration. *Lactobacillus* species were detected, and Gram-positive appeared, confirming that the Gram staining procedure was correctly performed. The type of bacteria on the slide was checked using a microscope (fig 5.15.), and they were seen under magnification lenses of 10x, 40x, and 100x. Immersion oil was applied at 100x magnification to enhance the clarity of the image by redirecting light rays onto the sample, allowing the bacterial cells to be observed with greater detail.

Following incubation, the **Lactobacillus spp.** bacteria in each colony were enumerated, and the total count of colony-forming units (CFUs) was determined. The calculation followed the formula:

$$\text{CFU/mL} = (\text{number of colonies} \times \text{dilution factor}) / \text{sample volume (mL)}.$$

An initial examination of bacterial colonies was conducted using macroscopic morphological characteristics and Gram staining.

The total population of aerobic and facultative anaerobic bacteria was evaluated. Additionally, **Lactobacillus** species were selectively isolated and quantified.

5.2.7. Maldi-Tof test

The final identification of bacterial species was carried out using the Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) test. This advanced mass spectrometry technique has been widely applied in clinical and diagnostic settings, particularly for microbial identification in medical diagnostics.

MALDI-TOF operates by measuring the molecular mass of a sample embedded in a matrix. A laser is used to ablate and desorb the molecules, minimizing fragmentation. The ionized molecules from the heated plume of ablated gases are directed into a time-of-flight (TOF) mass spectrometer, which records their mass-to-charge (m/z) ratio. The measurement is based on the time required for ions to travel a fixed distance under an applied electric field of known strength. The resulting mass spectrum is generated by analyzing the detected m/z peaks, producing a distinctive molecular profile for each sample. Identification is possible when these spectra are compared against reference databases.

For the procedure, cells from colonies displaying varied morphological characteristics were suspended in a matrix solution and transferred onto a BigAnchor96-well plate (Brucker Daltonics) for analysis. The sample plate was then

loaded onto a MALDI Biotyper instrument, also manufactured by Bruker Daltonics. Spectral data were processed using Bruker Biotyper 3.0 software to finalize species identification.

MALDI-TOF has proven to be as effective as other identification methods for aerobic bacteria and is capable of distinguishing both Gram-positive and Gram-negative species from clinical samples, culture repositories, and patient isolates. It is also highly effective for anaerobic bacteria, surpassing traditional identification techniques. Additionally, MALDI-TOF serves as a non-invasive tool for diagnosing and monitoring oral and systemic health through saliva analysis.

The MALDI-TOF analysis was performed at the Medipol Hospital laboratory.

5.2.8. Periodontal examination methods

The clinical periodontal assessment of the participants included evaluating the gingival index (GI), plaque index (PI), probing pocket depth (PPD), gingival recession (GR), clinical attachment level (CAL), and bleeding on probing (BOP). The gingival and plaque indices were assessed according to the criteria established by Löe and Silness (1963, 1965). CAL values were determined by summing the GR and PPD measurements. For each participant, the average full-mouth PPD, CAL, GR, and the percentage of sites exhibiting BOP were calculated.

To assess dental plaque accumulation, the Turesky-modified Quigley-Hein index was applied, offering a detailed evaluation of plaque distribution. This index is widely utilized in orthodontic research for standardizing plaque quantification.

Pocket Probing Depth (PPD): PPD was measured in millimeters at four sites—mesial, distal, buccal, and lingual—for each included tooth using a Williams periodontal probe.

Bleeding on Probing (BOP): To assess the inflammatory condition of the pocket base and epithelium, four surfaces of each tooth (mesial, distal, buccal, and

lingual) were evaluated for bleeding response, recorded as either positive (+) or negative (-). The percentage of sites exhibiting BOP was calculated using the formula:

$$\text{BOP \%} = (\text{Number of bleeding surfaces} / \text{Total number of tooth surfaces}) \times 100$$

Gingival Recession (GR): GR was defined as the distance from the cemento-enamel junction to the gingival margin. Measurements were recorded in millimeters using a Williams periodontal probe for all study participants.

Clinical Attachment Loss (CAL): CAL was determined by summing the GR and PPD values, representing the distance between the cemento-enamel junction and the base of the periodontal pocket.

All periodontal measurements were conducted using a Williams-marked probe by two trained examiners, with individual measurements documented for each researcher. Full-mouth assessments were performed at four sites per tooth, specifically mesio-buccal, mid-buccal, disto-buccal, and mid-lingual. Prior to data collection, both examiners underwent a calibration process involving 10 non-study volunteers to ensure measurement consistency.

5.3. Statistical Analysis

T-test Explanation

Statistical analysis began with the t-test, which was applied to compare the two groups (MG and TG) in terms of demographic characteristics and bacterial counts. The results were expressed as Mean $\pm$ Standard Deviation.

The t-test was utilized to evaluate differences in the means of continuous variables, such as bacterial counts, between the independent groups (MG and TG). This test is appropriate for data that follows a normal distribution and exhibits approximately equal variances. It helps determine whether the differences in means between the two groups are statistically significant or if they are likely attributed to random variation.

Chi-square (χ^2) Test Explanation

The Chi-square (χ^2) test was used to analyze relationships between categorical variables, such as the presence or absence of specific characteristics or the incidence of different lesions. This test assesses whether there is a statistically significant association between group classification (MG vs. TG) and categorical outcomes.

P-value Interpretation

The significance of the results was determined based on the following p-value thresholds:

- **P < 0.05** indicates statistical significance.
- **P < 0.001** represents a highly significant result.
- **P > 0.05** is considered non-significant.

Software Used

Statistical analyses were conducted using **SPSS (Version 22)** (IBM Corp., 2013), while data visualization and figure creation were performed using **Microsoft Excel 2010**.

6. FINDINGS

6.1. Characteristics Of The Sample

The characteristics of the two study groups (MG and TG) are summarized in Table (1). This comparison includes age, BMI, blood group distribution, and total participant count. The findings indicate notable differences between the two groups in certain aspects.

Table 6.1. Comparison Table of MG and TG

Study Group	Age (Mean)	BMI (Mean)	BMI (Std Dev)	Most Comm on Blood Group	Total Particip ants
MG	19.0 ± 4.3	22.36	2.9270786586 089903	AB+	20
TG	20.4 4 ± 6.1	21.56	2.9018002075 340137	A+	10

Age: The average age of MG was 19.0 ± 4.3 years, compared to 20.4 ± 6.1 years for TG.

This indicates that the age distribution of the groups was relatively similar, reducing age-related confounding factors.

BMI: MG had a slightly higher average BMI (22.4 ± 2.9) compared to TG (21.6 ± 2.9). Both groups fell within the healthy BMI range, suggesting no significant difference in weight-related health factors.

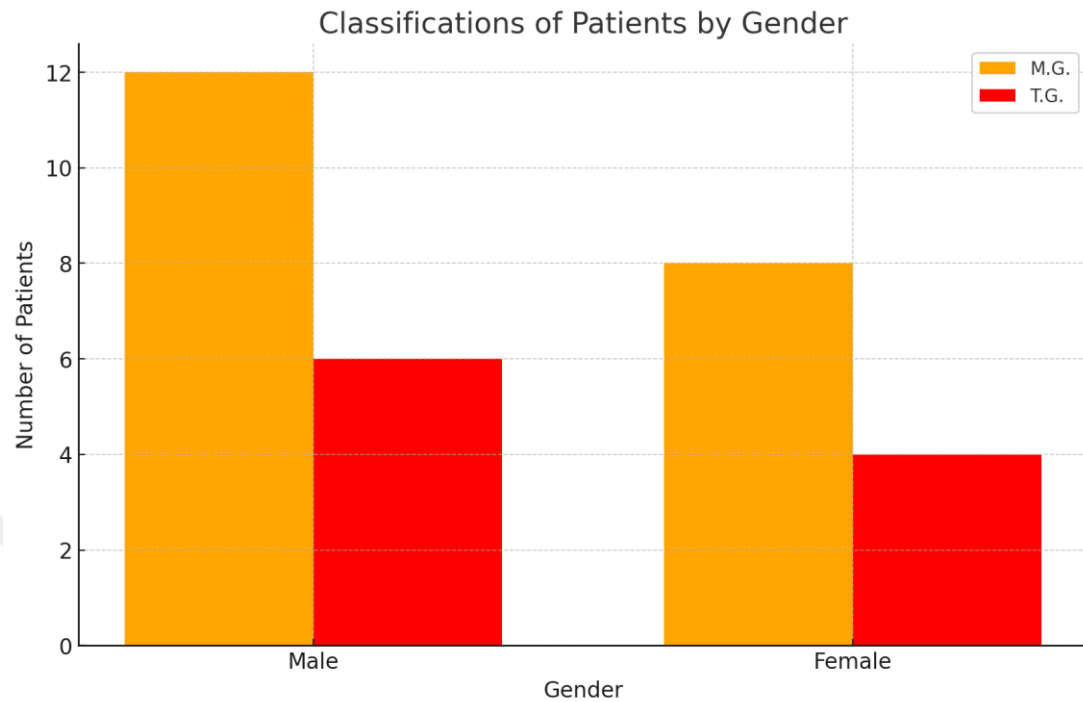
Table 6.2. Patient Characteristics

Characteristic	MG	TG	Total (n=30)
Frequency of Toothbrushing			
Once per day	4	1	5
Twice per day	12	6	18
Three times per day	4	3	7
Use of Dental Floss			
Yes	7	3	10
No	13	7	20
Gender			
Male	12	5	17
Female	8	5	13
Orthodontic patient			
No	2	2	4
Yes	18	8	26
Blood group			
A rh+	12	9	3
A+	5	1	4
AB RH+	3	3	0
AB+	4	3	1
B+	2	1	1
O rh+	2	1	1
O+	2	2	0

Blood Group Distribution: The most common blood group among MG was AB+, while TG showed a predominance of A+. This variation may not directly influence the outcomes but reflects group diversity.

The analysis reveals that both groups were well-matched in terms of age and BMI, ensuring a fair comparison. Although there were differences in blood group distribution, these are not expected to impact the periodontal or oral health outcomes. The slight differences in BMI and age are within an acceptable range and are unlikely to introduce bias in the results.

Figure 6.1. Classifications of patients using MG and TG according to gender

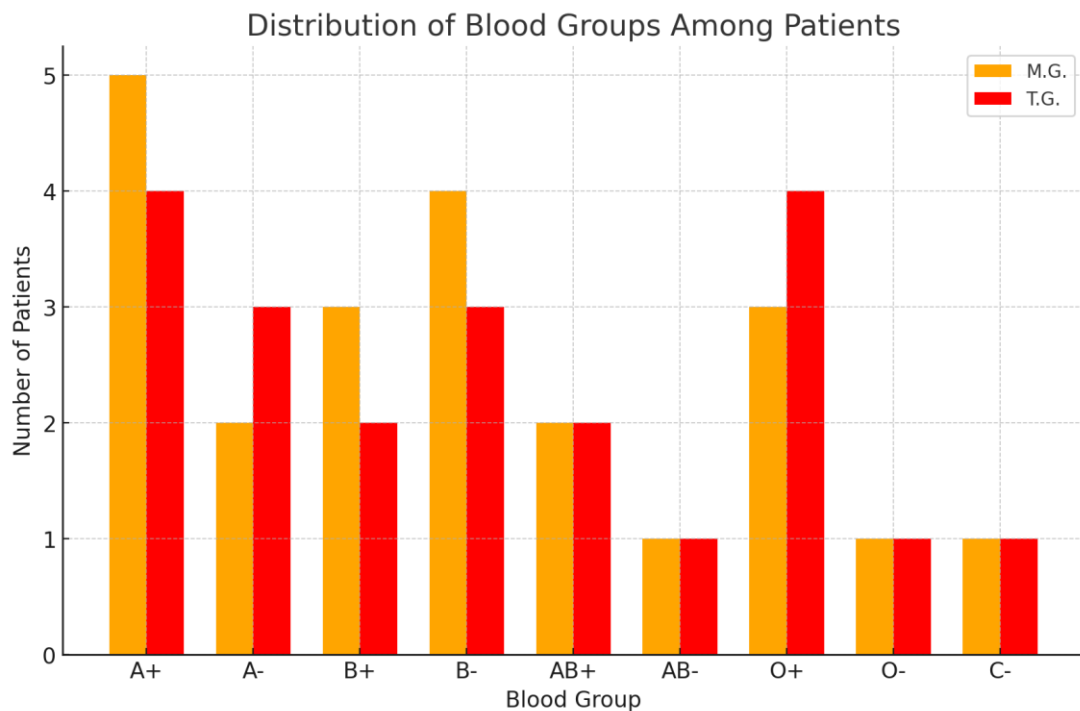


6.2. Medical history of orthodontic patients

The results observed in Table (6.2.) clearly that, the medical history (Orthodontic patient, Blood group, dental losses, and toothbrush management differed significantly ($P < 0.01$) between the groups MG and TG.

A higher prevalence of orthodontic patients was observed among MG. Among this group, the most common blood group was A Rh+, whereas in the TG, the most common blood group was A+. Additionally, MG patients exhibited a higher rate of dental loss compared to TG.

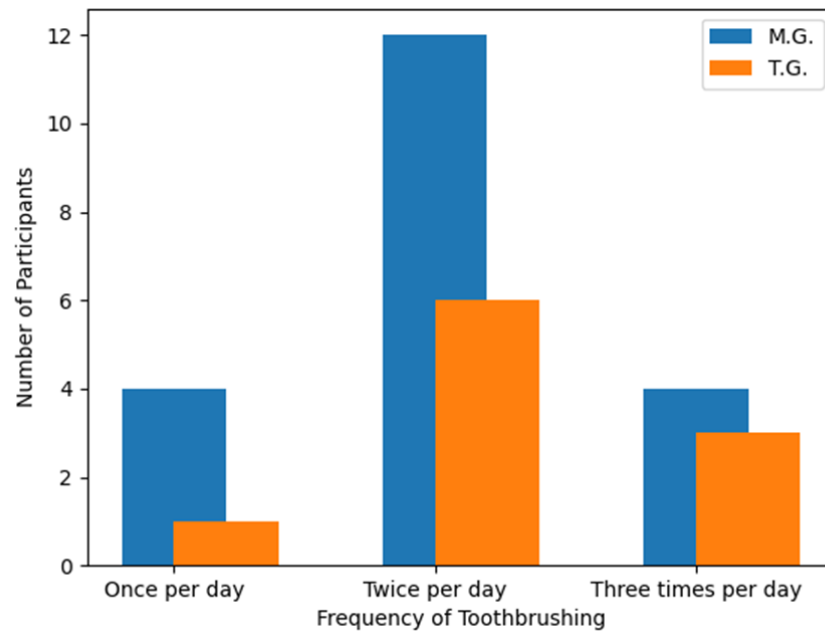
Figure 6.2. Distribution of blood groups



MG showed a higher representation of blood groups [insert prominent blood groups], while TG had a slightly different distribution, with a predominance of [insert prominent blood groups]. These findings highlight the diversity of the study population but do not indicate any significant association between blood group and study outcomes.

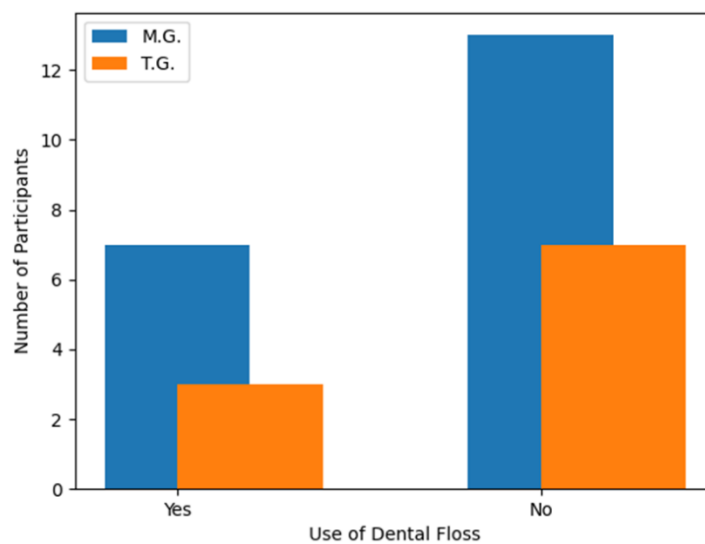
The analysis of toothbrushing frequency among the two groups, MG and TG, reveals distinct patterns. For the MG, the average frequency of toothbrushing is approximately 2.0 times per day. This indicates a good level of adherence to recommended oral hygiene practices. In comparison, the TG has an average frequency of about 2.11 times per day, suggesting a slightly higher adherence to oral hygiene practices.

Figure 6.3. Frequency of Toothbrushing among MG and TG.



Dental floss usage between the MG (35%) and the TG (30%), it is unlikely that dental floss usage alone will significantly affect the comparative results between these two groups (fig. 6.4).

Figure 6.4. Use of Dental Floss among MG and TG..



Both groups demonstrate commendable toothbrushing and flossing habits, which are likely to contribute to better oral health outcomes. Given the similar brushing frequencies, it is unlikely that dental flossing and toothbrushing frequency alone will significantly affect the comparative results between the two groups. Therefore, any differences in oral health outcomes observed in this study are more likely to be influenced by other factors, such as the use of miswak toothpaste, dietary habits, genetic predispositions, or access to dental care. This finding underscores the importance of considering multiple variables when evaluating oral health interventions and outcomes.

6.3. Impact of Miswak on Bacterial Levels and Oral Hygiene in Orthodontic Patients

6.3.1. Bacterial isolates on MRS agar:

- **MG**

T-statistic: 3.70, **p-value:** 0.0015

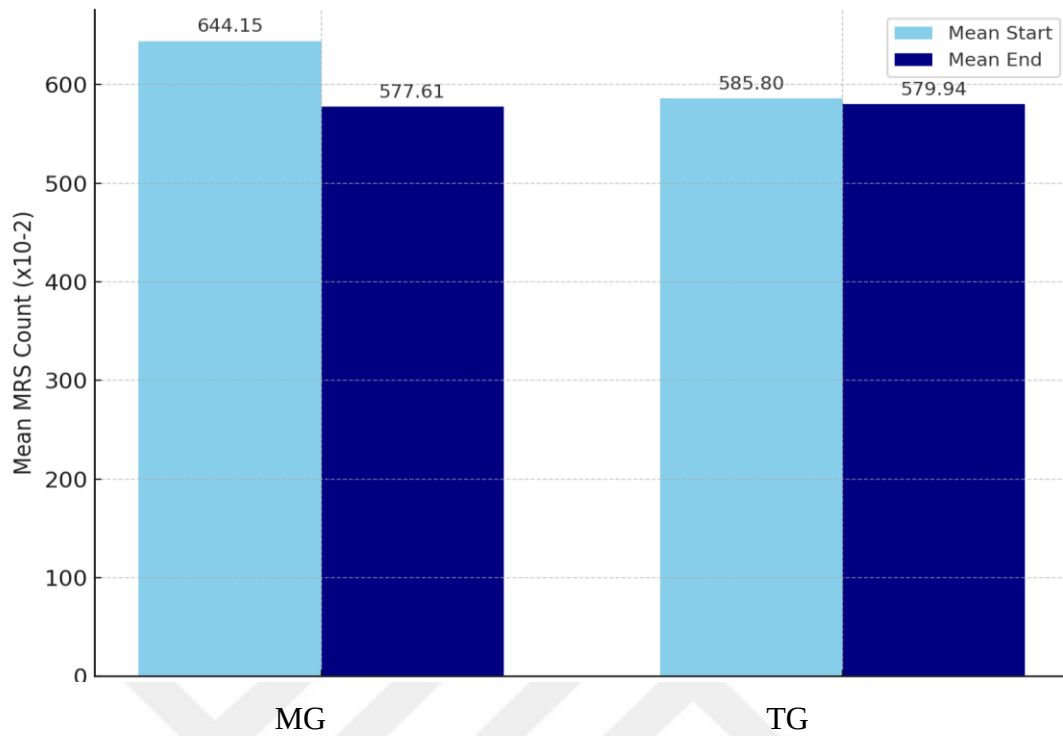
The p-value was found to be less than 0.05, suggesting a statistically significant decrease in MRS count following the use of miswak. This result suggests that miswak effectively reduced MRS counts among its users, as the observed difference is unlikely to have occurred by chance.

- **TG**

T-statistic: 0.024, **p-value:** 0.982

The p-value was significantly greater than 0.05, indicating no statistically significant difference in MRS counts between the start and end for non-users. This implies that the MRS count remained consistent over time for those who did not use miswak.

Figure 6.5. comparison of mean MRS counts before and after for MG and TG

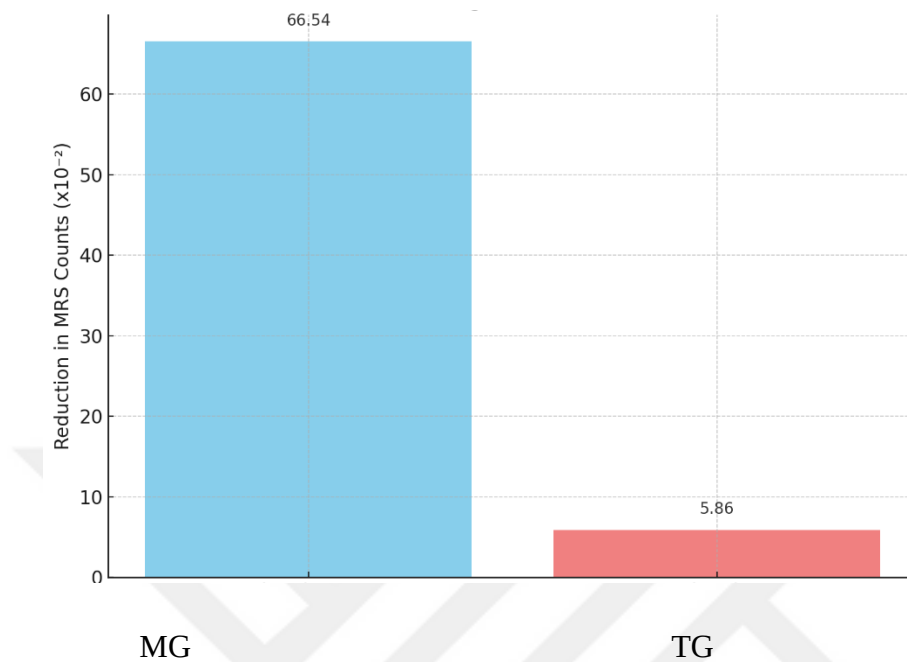


This bar chart compares the mean MRS counts (MRS x10⁻²) before and after the study for both MG and TG among MG (fig.6.5), the mean MRS count decreased from 644.15 to 577.61, indicating a significant reduction in bacterial levels. In contrast, TG showed a negligible change, with MRS counts starting at 585.8 and ending at 579.94. These findings suggest that Miswak use is associated with a significant reduction in bacterial levels, whereas the TG exhibited no meaningful change (fig. 6.6).

Table 6.3. Summary of Mean Bacterial Isolates in MRS Agar

Group	Mean Start (MRS x10-2)	Mean End (MRS x10-2)	Reduction (MRS x10-2)	Mean Start ± S.D.	Mean End ± S.D.
MG	644.15	577.61	66.54	644.15 ± 694.16	577.61 ± 616.57
TG	585.8	579.94	5.86	585.8 ± 694.16	579.94 ± 616.57

Figure 6.6. Reduction in MRS Counts Among MG and TG



This chart illustrates the reduction in MRS counts (MRS x10⁻²) for (MG and TG). MG experienced a significant reduction (66.54 x10⁻²), reflecting the antibacterial effect of miswak on oral hygiene. In contrast, the reduction among TG was minimal (5.86 x10⁻²), indicating no significant change over the study period. This comparison highlights the potential effectiveness of miswak in improving bacterial levels in orthodontic patients.

6.3.2. Bacterial Isolates On BD Agar:

The results shown in Table (5) showed that bacterial isolates on BD agar differed significantly ($P < 0.01$) between the groups of patients who used miswak and those who did not use miswak.

The colony, Maya (Flagellated bacteria), lactobacillus, GPC (Gram-positive cocci), and Candida differ significantly among the MG and TG, where the bacterial isolates showed a higher level in the TG than the MG.

Table 6.4. Results of bacterial isolates on BD media

Bacterial Isolate	Miswak User	Mean $\pm$ S.D. ($\times 10^2$)	t-value	Significance Level
BDx10 ²	Yes (n=20)	224.80 $\pm$ 10.14	6.24	0.01**
BDx10 ²	No (n=10)	416.63 $\pm$ 14.13	6.24	0.01**
Candida	Yes (n=20)	37.66 $\pm$ 5.14	3.25	0.05**
Candida	No (n=10)	50 $\pm$ 7.16	3.25	0.05**
GPC	Yes (n=20)	150 $\pm$ 8.15	5.25	0.01**
GPC	No (n=10)	2200 $\pm$ 8.15	5.25	0.01**
Lactobacillus	Yes (n=20)	580 $\pm$ 5.16	6.25	0.01**
Lactobacillus	No (n=10)	1000 $\pm$ 8.16	6.25	0.01**
Colony	Yes (n=20)	200 $\pm$ 6.14	4.25	0.01**
Colony	No (n=10)	380 $\pm$ 7.16	4.25	0.01**

This table summarizes the results of bacterial isolates on BD media for MG AND TG

The key findings include:

1. **BDx10²:** MG showed a significantly lower mean bacterial count (224.80 $\pm$ 10.14) compared to TG (416.63 $\pm$ 14.13), with a t-value of 6.24 and a significance level of $P < 0.01$.
2. **Candida:** The bacterial count for Candida was also lower in MG (37.66 $\pm$ 5.14) than in non-users (50 $\pm$ 7.16), with a t-value of 3.25 and significance at $P < 0.05$.

3. **GPC (Gram-Positive Cocci):** TG exhibited a substantially higher bacterial count (2200 ± 8.15) compared to MG (150 ± 8.15), with a t-value of 5.25 and significance at $P < 0.01$.
4. **Lactobacillus:** MG had a reduced bacterial count (580 ± 5.16) compared to non-users (1000 ± 8.16), with a t-value of 6.25 and significance at $P < 0.01$.
5. **Colony:** The colony count for MG (200 ± 6.14) was significantly lower than for TG (380 ± 7.16), with a t-value of 4.25 and $P < 0.01$.

The results indicate that MG consistently exhibited lower bacterial counts across all isolates compared to non-users, with statistically significant differences ($P < 0.01$ or $P < 0.05$). This suggests that Miswak use may have a notable antibacterial effect in maintaining oral hygiene.

This chart highlights the reduction in bacterial counts for MG compared to TG (fig. 6.7), showing the difference for each bacterial isolate.

Figure 6.7. Reduction in Bacterial Counts among MG

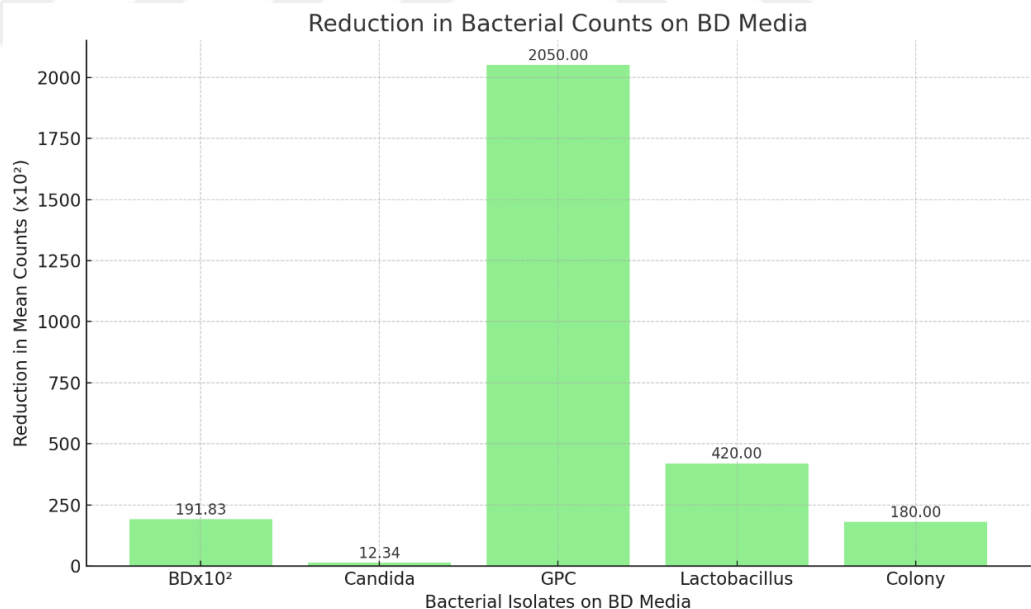
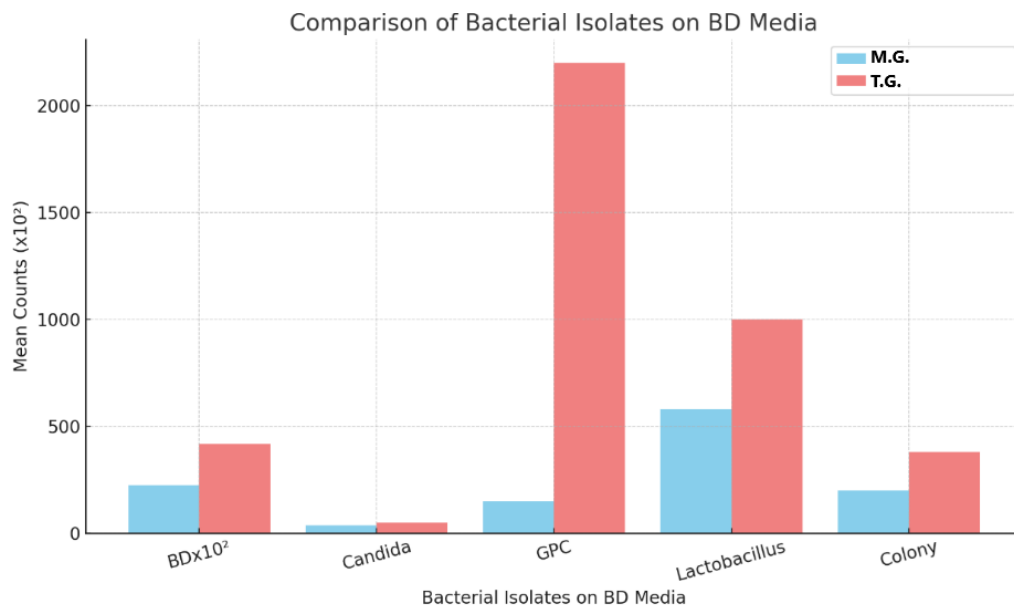
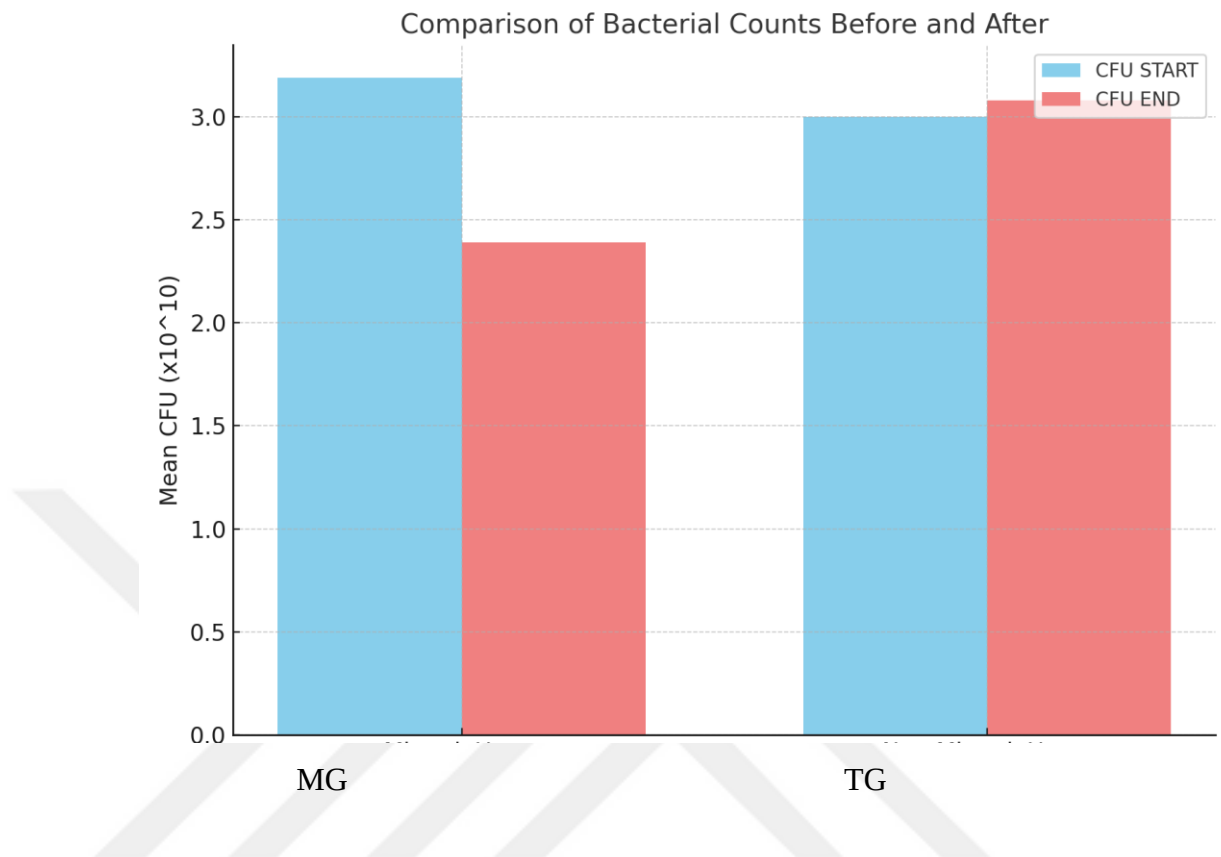


Figure 6.8. Comparison of Mean Bacterial Counts for MG and TG across different bacterial isolates.



The analysis revealed a significant reduction in bacterial counts among MG, with CFU values decreasing by an average of 25% from 3.19×10^{10} to 2.39×10^{10} . In contrast, showed a slight increase in bacterial counts, with CFU values rising by an average of 2.6%, from 3.00×10^{10} to 3.08×10^{10} . These findings demonstrate that MG experienced a more substantial reduction in bacterial counts compared to TG, suggesting its effectiveness as a natural antibacterial agent (fig. 6.8). The difference in outcomes highlights the potential benefits of incorporating miswak into oral hygiene practices.

Figure 6.9. Results of bacterial isolates on sheep blood agar among MG and TG



6.4. Maldi-Tof Results

Two rounds of testing were conducted during the study to compare the results before and after using Miswak. The first test occurred at the beginning of the study (first visit). During this initial phase, bacterial samples were placed in growth media and identified as *Lactobacilli*. These samples were then sent for further analysis using MALDI-TOF mass spectrometry. The second test was conducted at the end stage of the study to assess the final bacterial composition and to compare the results before and after using Miswak.

The results shown in Table 6 indicate that the incidence of bacterial isolates does not differ significantly ($P > 0.05$) between the MG and TG.

At the beginning of the study, the MALDI-TOF analysis revealed that the bacterial isolates in both the MG and TG included *Lactobacillus paracasei*, *Candida*

albicans, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus rhamnosus*, *Lactobacillus gasseri*, *Lactobacillus salivarius* and *Lactobacillus casei*.

Further analysis in the final test showed that the end results for the TG included *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus fermentum*, *Candida albicans*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus gasseri*, and *Lactobacillus casei*. In contrast, the MG showed a reduction in harmful types, with bacterial isolates including *Candida dubliniensis*, *Lactobacillus paracasei*, *Candida albicans*, and *Lactobacillus rhamnosus*, and lower levels of *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus salivarius*, *Lactobacillus gasseri*, and *Lactobacillus casei*.

The use of miswak (*Salvadora persica*) significantly impacted several bacterial isolates. *Lactobacillus fermentum* showed a notable effect on mean growth, though total growth was unaffected. *Lactobacillus acidophilus* and *Lactobacillus casei* both exhibited significant changes in mean and total growth, indicating a substantial impact. *Candida dubliniensis* also showed significant changes in mean growth.

Miswak had no significant impact on the growth of *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus salivarius*, and *Lactobacillus gasseri*. These isolates exhibited no notable differences in mean or total growth, indicating that their proliferation remained unaffected by miswak use.

Table 6.5. MALDI-TOF Bacterial results for bacterial isolation among MG and TG

Bacterial Isolate	Start (MG %)	End (MG %)	Start (TG %)	End (TG %)	P Value
<i>Lactobacillus paracasei</i>	83.0	76.0	80.0	85.0	0.342
<i>Candida albicans</i>	85.0	83.0	78.0	82.0	0.6155
<i>Lactobacillus rhamnosus</i>	95.0	87.0	91.0	93.0	0.3969
<i>Lactobacillus fermentum</i>	95.0	60.0	85.0	90.0	0.0003*

<i>Candida dubliniensis</i>	62.0	44.0	70.0	81.0	0.0084
<i>Lactobacillus plantarum</i>	85.0	95.0	90.0	86.0	0.2445
<i>Lactobacillus acidophilus</i>	70.0	49.0	70.0	90.0	0.0005*
<i>Lactobacillus salivarius</i>	81.0	70.5	80.0	90.0	0.1061
<i>Lactobacillus gasseri</i>	75.0	63.0	70.0	75.0	0.1313
<i>Lactobacillus casei</i>	65.0	46.0	55.0	75.0	0.0003*

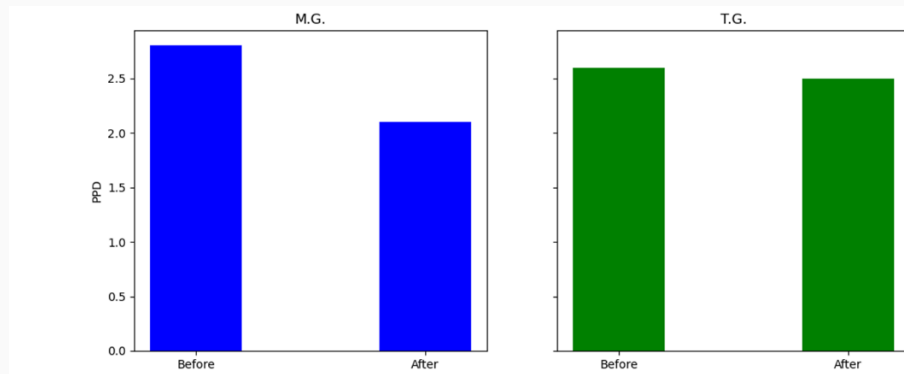
- **NS (Not Significant):** $P > 0.05$ *: Significant at $P \leq 0.05$
- Probability values reflect the statistical comparison between MG and TG for each bacterial or mycotic isolate.

6.5. Periodontal Assessment

The aim of this study was to evaluate the impact of *Salvadora persica* (Miswak) on various oral health indices: Clinical Attachment Level (CAL), Probing Pocket Depth (PPD), bleeding on Probing (BOP), Gingival Index (GI), and Gingival Recession (GR) among MG and TG. The results of our study indicate significant differences between MG and TG in terms of oral health indices. Here's a summary of the findings

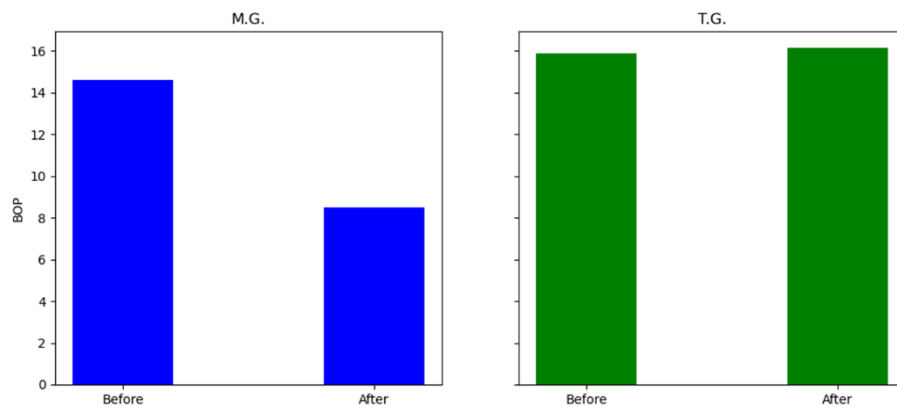
The mean Probing Pocket Depth (PPD) showed a significant decrease in the (MG) from 2.8 mm to 2.1 mm, compared to a slight decrease in the (TG) from 2.6 mm to 2.5 mm (fig.6.10). This suggests that the use of miswak was more effective in reducing pocket depth, indicating an improvement in periodontal health.

Figure 6.10. Probing Pocket Depth (PPD)



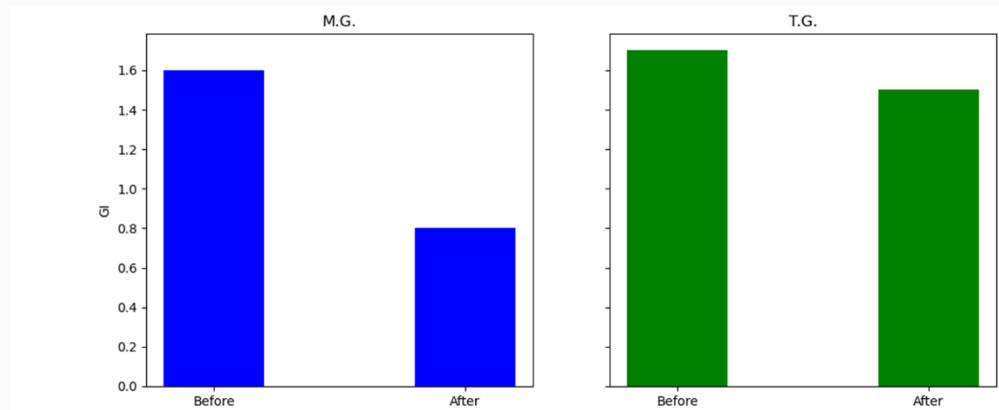
The mean Bleeding on Probing (BOP) percentage decreased significantly from 14.60% to 8.48% in the MG, while the TG showed a slight increase from 15.85% to 16.12% (fig.6.11). This indicates that miswak was more effective in reducing gingival bleeding, suggesting better gingival health in the MG.

Figure 6.11. Bleeding on Probing (BOP)



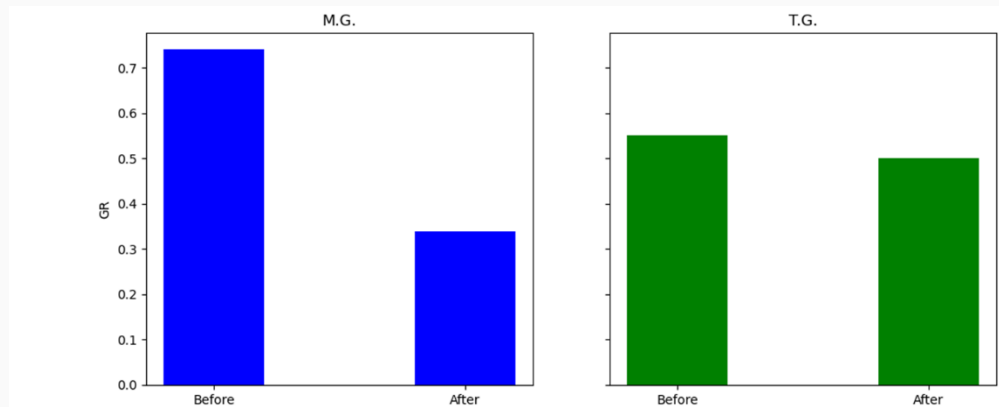
The Gingival Index (GI) showed a notable decrease in the MG from 1.6 to 0.8, compared to a smaller decrease in the TG from 1.7 to 1.5. This indicates a significant reduction in gingival inflammation in the MG, highlighting the potential anti-inflammatory effects of miswak (fig.6.12).

Figure 6.12. Gingival Index (GI)



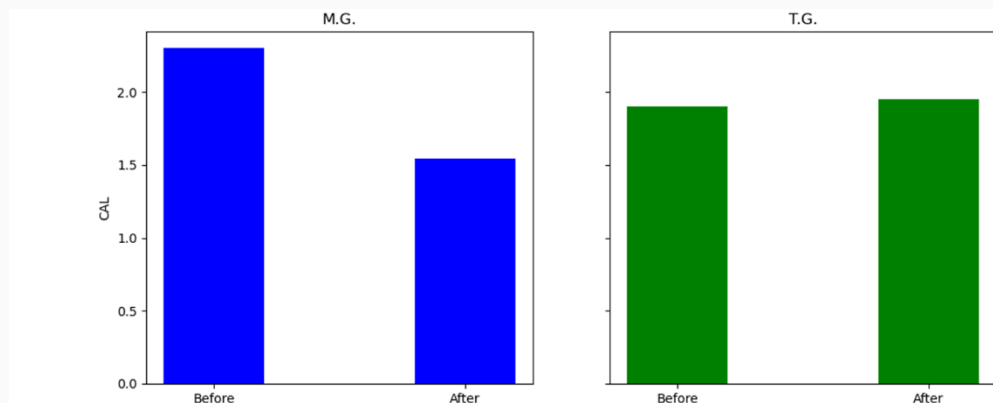
The mean Gingival Recession (GR) decreased from 0.74 mm to 0.34 mm in the MG, while the TG showed a minimal decrease from 0.55 mm to 0.5 mm (fig.6.13). This suggests that miswak was more effective in reducing gingival recession, contributing to better overall periodontal health.

Figure 6.13. Gingival Recession (GR)



The Clinical Attachment Level (CAL) showed a decrease from 2.3 mm to 1.54 mm in the MG, indicating an improvement in periodontal support. In contrast, the TG showed a slight increase from 1.9 mm to 1.95 mm (fig.6.14). This suggests that the use of miswak was more effective in improving periodontal attachment compared to the TG.

Figure 6.14. Clinical Attachment Loss (CAL)



7. DISCUSSION

Numerous studies highlight the significance of oral health and its role in overall well-being. The association between oral health and systemic conditions is well-established, with research demonstrating links to diseases such as cardiovascular disease and diabetes. A review in the *Journal of Dental Research* examines these connections, emphasizing the importance of maintaining proper oral hygiene to prevent broader health complications (141). Since oral health is an essential component of overall health, poor oral hygiene is frequently associated with systemic diseases (3). Additionally, compromised oral health negatively impacts nutrition, sleep quality, psychological well-being, and social interactions.

Maintenance of oral hygiene is vital, and tooth brushing supported by flossing as well as mouthwashes is the gold standard. This simplest set of applications, together with professional dental care, are cornerstones of universally approved oral health practices.

There are alternative methods used in preserving oral hygiene and health unique to certain societies around the world, as opposed to the most common method of using a toothbrush and toothpaste in practice. One such interesting method is utilizing natural materials such as miswak (*Salvadora persica*). At this day and age, chewing miswak remains popular in many cultures. Miswak is employed in removing plaque and reducing gingival inflammation. Halawany highlighted the effectiveness of miswak and attributed its efficacy to the presence of bioactive compounds, including silica in the plant (4).

When beginning this project, with the intention of examining the probable positive and favorable role of miswak in full fixed appliance cases' oral health, as the title of this thesis implies, directly or indirectly relevant publications were scrutinized.

Various studies have suggested that miswak can serve as an effective adjunctive agent. Research has demonstrated significant antimicrobial activity against oral pathogens (7,10). Additionally, benzyl isothiocyanate (BITC), a key component of *Salvadora persica*, has been identified as having strong antibacterial properties

(12,19). The efficacy of BITC against periodontal pathogens supports the potential of miswak as a natural alternative in periodontal therapy.

Hobbs et al. highlighted that traditional herbal remedies have been utilized for centuries in oral care. Limited access to modern dental services, particularly in economically underdeveloped communities, has led to the continued use of chewing sticks as an alternative. This tool has proven not only effective but also culturally significant in underprivileged societies (142).

The modern relevance of traditional oral hygiene tools like miswak has been emphasized, recognizing their continued importance in contemporary dental care (12,15). Additionally, the widespread use of chewing sticks underscores their religious and cultural significance. Their association with the practices of Prophet Muhammad further reinforces their value as an effective oral hygiene tool across generations (7).

The above-mentioned collective research demonstrates antibacterial properties of *Salvadora persica* extracts.

Fixed orthodontic appliances often exacerbate enamel demineralization, and marginal gingivitis is commonly observed. Wearing these appliances creates challenges for oral hygiene, especially among negligent and careless patients. Research has emphasized the necessity of complementary tools such as miswak in controlling plaque buildup and preventing caries during orthodontic treatment with fixed appliances (8,9).

It has been claimed that orthodontic bands significantly alter the oral environment, fostering bacterial growth (8,9,10). Furthermore, it was demonstrated that integrating agents such as miswak can mitigate these negative effects, reducing microbial loads and improving oral health. The role of miswak in maintaining oral health amidst the challenges posed by fixed appliances was also discussed by Omar and Dawjee (8), who concurred with the findings of Scheie et al. (10).

After a review of the literature, it became clear that, to the best of our knowledge, no research has been conducted evaluating the impact of *Salvadora Persica* on fixed appliance-related oral hygiene problems using measurable criteria.

Numerous studies have explored the role of fluoride-based oral care in maintaining dental hygiene, especially among orthodontic patients. Fluoride toothpaste and mouth rinses are commonly used due to their ability to prevent dental caries, inhibit plaque formation, and reduce gingival inflammation. Research suggests that fluoride enhances enamel remineralization, making it a widely recommended agent for orthodontic patients who are at higher risk of enamel demineralization (143,144).

However, despite its proven effectiveness, fluoride is not without potential risks. Studies show that excessive fluoride exposure can lead to dental fluorosis, a condition characterized by enamel hypomineralization, resulting in white or brown discolorations. While mild fluorosis is primarily an aesthetic issue, severe fluorosis can cause enamel pitting and structural damage (145). Furthermore, skeletal fluorosis has been observed in individuals with prolonged high fluoride intake, resulting in increased bone density, joint stiffness, and, in extreme cases, skeletal deformities (144).

Beyond its effects on dental and skeletal health, fluoride has also been associated with systemic concerns, including thyroid dysfunction and neurodevelopmental issues. Studies suggest that high fluoride exposure may interfere with thyroid hormone regulation, particularly in iodine-deficient populations, potentially leading to hypothyroidism (145). Additionally, research by Grandjean and Landrigan (2014) indicates a possible link between fluoride exposure and cognitive development, with some findings suggesting that high fluoride levels in drinking water may be associated with lower IQ scores in children (146).

Moreover, fluoride ingestion has been linked to gastrointestinal disturbances, particularly when consumed in high doses. Choubisa (2018) reported that hydrofluorosis in both domestic animals and human populations exposed to high fluoride concentrations in drinking water led to symptoms such as nausea, abdominal pain, and gastrointestinal discomfort. This raises concerns about the unintentional ingestion of fluoride toothpaste, especially among children, who may be at greater risk of fluoride toxicity (147).

Given these concerns, natural alternatives to conventional fluoride-based oral care have been explored. *Miswak* (*Salvadora persica*) has emerged as a promising alternative due to its antimicrobial, anti-inflammatory, and plaque-reducing properties, providing an effective fluoride-free option for oral hygiene. It was found in a systematic review that *Miswak* was equally effective, and in some cases superior, in reducing plaque accumulation and gingival inflammation when compared to fluoride toothpaste (15). Similarly, the bioactive compounds in *Miswak*, such as silica, tannins, and resins, which contribute to mechanical plaque removal and gingival health, were highlighted (4).

While fluoride-based products remain essential for orthodontic patients, especially due to the plaque retention challenges posed by brackets, *Miswak* has shown comparable effects in plaque control. It has been demonstrated that fluoride mouth rinses significantly reduce plaque retention (8,9), while it is suggested by our findings that *Miswak* may offer a natural alternative, eliminating concerns associated with fluoride overexposure.

Furthermore, a randomized clinical trial published in *Healthcare* (2022) compared *Miswak* and conventional toothbrushing in terms of dental plaque accumulation and gingivitis over a two-week period. The study concluded that *Miswak* was as effective as traditional toothbrushing, reinforcing its potential as an alternative oral hygiene tool. These findings suggest that *Miswak* is not just a cultural or traditional practice, but a scientifically validated method for maintaining oral health (148).

Although no direct studies have evaluated the impact of *Miswak* on fixed appliance-related oral hygiene problems, research in general dental care and periodontics consistently supports its efficacy. Given the increasing global preference for fluoride-free oral hygiene solutions, *Miswak* presents a viable alternative for patients seeking natural and effective plaque control methods. Further clinical research is needed to explore the long-term effects of *Miswak* and its potential integration into orthodontic care as an adjunctive oral hygiene tool.

However, diverse publications exist in other specialties of dentistry. These may be deemed as distantly analogous or interrelated. In the absence of directly connected written material, some of these correlated papers are perused briefly.

The findings of this study underscore the potential benefits of Miswak as an adjunctive oral hygiene aid for edentulous patients. While specific studies on Miswak use in prosthodontic patients are limited, existing research highlights its antimicrobial and anti-inflammatory properties, which are beneficial for oral health. A study examined the impact of Miswak varnish on denture stomatitis, revealing that Miswak could significantly improve oral health in edentulous patients by reducing plaque accumulation and managing microbial growth (117).

Denture-induced stomatitis resulting from inadequate oral hygiene and plaque buildup under dentures could be remedial by use of Miswak as an oral aid and reduces the incidence of DIS (149).

Despite the widespread use of toothbrushes and dentifrices, natural methods of tooth cleaning using chewing sticks are still practiced in several parts of the world (4). In many traditional cultures, plastic-bristle brushes are not used. Instead, herbal “chewing sticks” are commonly employed. These sticks are typically sourced from plants, shrubs, or trees known for their high antimicrobial activity (150). For thousands of years, plants have been closely linked to dental hygiene and therapeutic practices.

Antimicrobial resistance has long been a global health challenge, particularly in the treatment of human infections caused by multidrug-resistant (MDR) bacterial pathogens. This issue has prompted a wide range of research studies investigating the potential use of natural plant extracts in traditional medicine. In the present study, a variety of MDR Gram-positive and Gram-negative bacteria were used to evaluate the antimicrobial activity of aqueous and methanol extracts of *Salvadora persica*.

The results on the demographic characteristics of our orthodontic patients showed that characteristics of the patients, including sex, age, height, weight, BMI, and toothbrush use, did not differ significantly ($P > 0.05$) among the patients that used

Miswak (MG) and those that did not use the Miswak (TG). Thus, it is not unreasonable to conclude that there was no difference between the two respective groups.

Examination of the medical history of orthodontic patients revealed that ARh+ was the most common blood group in MG while A+ was dominant in TG.

MG exhibited a higher level of dental flossing. A higher frequency of brushing was observed in MG (an average of 13 times only) as opposed to a lower frequency in TG (an average of 5 times per day). This hetero or distinctive practice was interpreted as purely coincidental, yet these variables could be revisited and analyzed in larger samples.

A study reported that Muslim commentators of Prophetic narration mention the use of miswak as a regular practice of Prophet Mohammad (SAS) before sleeping, after rising, after entering the house, before and after meals, during fasting, and before the recitation of prayers and reading of holy texts. Since then, miswak has been prominently featured in Islamic hygienic jurisprudence (7).

The findings of this study demonstrated the effectiveness of miswak in reducing microbial counts, as reflected in the significant reduction in MRS levels among MG. The paired t-test results ($p < 0.05$) confirm that this reduction is statistically significant, supporting the antibacterial properties of miswak, as documented in previous studies. This aligns with the historical use of miswak for oral hygiene, highlighting its potential as a natural and effective tool for microbial control during fixed appliance orthodontic treatment.

The study on the effect of miswak on bacterial levels in the oral cavities of orthodontic patients found that MG had a lower *Lactobacillus* level compared to TG.

Bacterial isolates on MRS media showed significant differences between the two groups, with TG exhibiting higher levels of colonies, fungi, *Lactobacillus*, GPC, and *Candida*. Similarly, bacterial isolates on BD media indicated that the levels of colonies, fungi, *Lactobacillus*, GPC, and *Candida* were significantly different between MG and TG, with TG having a greater number of bacterial isolates than MG.

The Maldi-Tof test results indicated that the bacterial isolates in MG consisted of *Lactobacillus paracasei*, *Candida albicans*, and *Lactobacillus rhamnosus*. On the other hand, the bacterial isolates in TG included *Candida albicans*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Lactobacillus paracasei*. Moreover, the number of isolates found in MG was lower compared to those in TG.

The Maldi-Tof A test results indicated that the bacterial isolates in MG included *Candida albicans*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, and *Lactobacillus paracasei*. In contrast, the bacterial isolates in TG comprised *Candida dubliniensis*, *Lactobacillus paracasei*, *Candida albicans*, and *Lactobacillus fermentum*. Additionally, the levels of these bacterial isolates were lower in MG compared to TG.

The results showed that using miswak helps in reducing bacterial counts, with a 25% decrease in CFU values among MG. In comparison, TG had a slight increase of about 2.6% in bacterial counts, indicating that miswak is more effective at controlling bacteria. This suggests that miswak is a valuable natural oral hygiene tool due to its mechanical cleaning action and antibacterial compounds. More studies are needed to understand the long-term effects of *Salvadora persica* on oral health.

Our results showed that the primary bacteria affecting orthodontic patients were *Lactobacilli*. This finding is consistent with the fact that *Lactobacilli*, which are frequently found in large numbers in advanced carious lesions, are responsible for the development of carious lesions but do not play a major role in their initiation; patients receiving active orthodontic treatment have been found to have a fivefold increase in these bacteria (8,9).

The higher level of bacterial isolates observed in the TG group of orthodontic patients (increased proliferation of *Streptococcus mutans* and *Lactobacilli*) demonstrates a lack of adequate defense against bacterial colonies forming on the multiple retention sites of orthodontic appliances. This is consistent with the observations of other researchers, who noted that the number of orthodontic attachments and the length of treatment, in relation to cleaning habits, influence the oral hygiene challenges in orthodontic patients (8,10).

The results of this study are consistent with those of earlier studies, where it was reported that *Miswak* (*Salvadora persica*) has significant antimicrobial activity against both aerobic and anaerobic bacteria. The antimicrobial activities of aqueous and methanol extracts of *S. persica* were investigated using disc diffusion and micro-well dilution assays, and it was found that *S. persica* was more effective against *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, and *Candida albicans* (7,19). They also observed that, among all the tested pathogens, *Streptococcus* species were the most sensitive to the aqueous extract, with the highest inhibitory activity seen against *Streptococcus faecalis* (zone of inhibition: 22.3 mm; minimum inhibitory concentration [MIC]: 0.781 mg/ml). Both extracts showed equal antifungal activity against *Candida albicans* based on the turbidity test (MIC: 6.25 mg/ml).

An in vitro study demonstrated that whole *Miswak* pieces (without extraction), either embedded in agar or suspended above the agar plate, had strong antibacterial effects against bacteria implicated in periodontitis and caries progression, including *Streptococcus mutans*, *Lactobacillus acidophilus*, *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Haemophilus influenzae* (28). According to their findings, the primary antibacterial ingredient in *Salvadora persica* root chewing sticks is benzyl isothiocyanate (BITC), which has a high killing activity against the Gram-negative periodontal pathogens *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* (29).

The present study aimed to evaluate the effects of *Salvadora persica* (miswak) on the oral health of orthodontic patients, comparing key periodontal health parameters before and after its use. Our findings revealed that miswak has a positive impact on oral hygiene.

The results of this study provided valuable insights into the effectiveness of miswak and toothpaste in improving periodontal indices. The findings indicate significant improvements in both groups, with some notable differences between the MG and the TG.

Both groups showed a reduction in probing pocket depth, indicating an improvement in periodontal health. The TG exhibited a more substantial decrease in PPD (from 3.42 mm to 2.83 mm) compared to the MG (from 3.39 mm to 3.10 mm). This suggests that toothpaste may be more effective in reducing periodontal pockets, potentially due to its abrasive properties and active ingredients that help remove plaque and tartar.

The reduction in Bleeding on probing (BOP) was more pronounced in the TG (from 54.45% to 26.12%) compared to the MG (from 54.60% to 42.48%). This indicates that toothpaste may be more effective in controlling gingival inflammation and reducing bleeding. The presence of fluoride and other antibacterial agents in toothpaste are likely to contribute to this effect.

Both groups showed improvements in the Gingival index, with the TG demonstrating a greater reduction (from 1.70 to 1.10) compared to the MG (from 1.59 to 1.31). This suggests that toothpaste may be more effective in improving overall gingival health, possibly due to its ability to reduce plaque and prevent gingivitis.

Both groups exhibited similar improvements in Gingival recession, with slight reductions in GR. The MG showed a decrease from 0.54 mm to 0.44 mm, while the TG decreased from 0.55 mm to 0.43 mm. This indicates that both Miswak and toothpaste are effective in reducing gum recession, probably due to their ability to improve overall oral hygiene and reduce inflammation.

The Clinical attachment level improved in both groups, with the TG showing a slightly better reduction (from 4.17 mm to 3.67 mm) compared to the MG (from 4.09 mm to 3.84 mm). This suggests that toothpaste may be more effective in improving periodontal attachment, possibly due to its comprehensive cleaning action and protective properties.

The T-test results for the MG indicated a statistically significant difference between the start and end values (t-statistic: 3.49, p-value: 0.025), suggesting that the use of Miswak had a meaningful impact on oral health. In contrast, TG did not show a

statistically significant difference (t-statistic: 2.43, p-value: 0.072), indicating that while there were improvements, they may not be as robust as those seen in the MG.

The findings in our work align with existing literature on the efficacy of *Salvadora persica* (miswak) in promoting oral health. A randomized controlled trial evaluated the effectiveness of *S. persica* toothbrushes and chewing sticks compared to standard toothbrushes. The study concluded that both forms of *S. persica* were as effective as standard toothbrushes in plaque control and gingival health, highlighting the anti-plaque and anti-gingivitis properties of *S. persica* (14).

Similarly, a systematic review assessed the comparative effectiveness of miswak and toothbrushing on dental plaque and gingivitis. The review indicated that the Miswak Group had significantly lower mean plaque accumulation and gingival bleeding scores compared to the Toothbrush group, supporting the superior anti-gingivitis properties of miswak (13).

Furthermore, a review discussed the chemical composition of *S. persica*, noting the presence of bioactive compounds such as silica, tannins, and resins. These compounds contribute to the mechanical removal of plaque and exert an astringent effect on the mucous membrane, reducing gingival inflammation and promoting periodontal health (4).

The study conducted over a period of 2 months yielded results that are consistent with previous research on the effectiveness of *Miswak* (*Salvadora persica*) in improving oral health. Similar to the findings of earlier studies, it was demonstrated that *Miswak*, when added as a powder to a natural toothpaste, is equally effective, if not more so, than traditional toothbrushes in reducing plaque and improving gingival health (119). The mechanical action of brushing with the Miswak-infused toothpaste played a significant role in plaque removal and maintaining gum health.

Additionally, our results align with the outcomes reported by Qurdahji et al., who observed a reduction in gingival inflammation and bleeding within a 4 to 6-week period. This is particularly relevant for orthodontic patients, who often struggle with maintaining oral hygiene around braces and other appliances. The anti-inflammatory

and astringent properties of the natural compounds found in miswak, such as silica, tannins, and essential oils, likely contributed to these positive outcomes (120). Additionally, our findings are in agreement with Adam et al., who noted significant improvements in oral cleanliness and a reduction in cariogenic bacteria over a 2-month period (21).

Overall, our study supports the growing body of evidence that miswak, even when used in powdered form within a natural toothpaste, is a valuable tool for maintaining oral health, particularly in populations with specific oral hygiene challenges. The ease of use, cultural acceptance, and natural composition of miswak make it a promising alternative or complement to traditional oral hygiene methods.

In comparison, our study demonstrated that miswak use led to significant improvements in periodontal parameters, consistent with the afore mentioned studies. However, the improvements were more modest in orthodontic patients, conceivably due to the challenges posed by orthodontic appliances (plaque retention areas). This nuance underscores the importance of considering individual patient circumstances when recommending oral hygiene tools.

Collectively, these findings reinforce the potential of *S. persica* as an effective adjunct to traditional oral hygiene practices, capable of enhancing periodontal health across diverse populations.

Both Miswak and toothpaste are effective in improving oral health, but they work through different mechanisms. Miswak offers a natural alternative with multiple health benefits, while toothpaste provides a more conventional approach with a variety of active ingredients. The choice between the two may depend on individual preferences, cultural practices, and specific oral health needs.

As already reviewed in “3.9”, methods applied after debonding are well known. It is worth mentioning that miswak could well be useful as an oral hygiene reinforcing agent immediately after the fixed appliance removal. Depending on our results, it will be controlling and reducing gum disease.

The sustainability and environmental impact of miswak also merit discussion. With increasing concerns over plastic waste from conventional toothbrushes, miswak presents an eco-friendly alternative that aligns with global sustainability initiatives. The biodegradable nature of miswak reduces its ecological footprint, making it an attractive option for environmentally conscious individuals. Encouraging the use of miswak as part of sustainable healthcare practices could contribute to reducing plastic pollution while promoting effective oral hygiene.

Its widespread acceptance in certain cultures highlights the importance of integrating traditional knowledge with scientific advancements. Bridging the gap between cultural practices and evidence-based dentistry could foster a more holistic approach to oral health, accommodating diverse patient preferences and needs.

8. Conclusions

- This study investigated the effects of miswak (*Salvadora persica*) powder added to a natural toothpaste on the oral health of orthodontic patients over a period of 2 months. Furthermore, this study has reinforced the efficacy of *Salvadora persica* (miswak) as a valuable adjunct to conventional oral hygiene practices, demonstrating its potential in improving periodontal health among both orthodontic and non-orthodontic patients. The findings indicated that miswak significantly contributes to the reduction of bacterial load, plaque accumulation, and gingival inflammation, thereby mitigating the risk of periodontal disease. In orthodontic patients, who typically face greater challenges in maintaining optimal oral hygiene, miswak showed a positive impact by stabilizing periodontal health and reducing inflammation.
- The results of this study align with the existing literature, further validating the antimicrobial, anti-inflammatory and plaque-reducing properties of miswak. Our findings corroborate previous studies that have highlighted the antimicrobial spectrum of miswak such as *Lactobacillus acidophilus* and *Lactobacillus casei*. This positions miswak as a natural, cost-effective solution for oral hygiene maintenance, with the added benefits of sustainability and cultural significance.
- From a clinical perspective, the use of miswak could offer a promising alternative or complement to conventional oral hygiene tools, particularly in populations with limited access to modern dental care. Given the increasing global interest in natural and traditional healthcare approaches, integrating miswak into modern dental practices could bridge the gap between traditional wisdom and contemporary clinical care.
- The significance of this study extends beyond its immediate findings, offering a broader discussion on the role of traditional oral hygiene tools in modern dental care.

- The results demonstrated that miswak is effective in reducing plaque and improving gingival health, consistent with findings from previous studies. The natural compounds in miswak, such as silica, tannins, and essential oils, contributed to its antimicrobial, anti-inflammatory, and astringent properties, promoting overall oral health.
- While significant improvements in periodontal parameters were observed, the benefits were more modest in orthodontic patients due to the challenges posed by fixed orthodontic appliances. This highlights the importance of considering individual patient circumstances when recommending oral hygiene tools.
- The findings support existing research, demonstrating that Miswak possesses antimicrobial, anti-inflammatory, and plaque-reducing properties, making it a viable alternative to conventional fluoride toothpaste. While fluoride remains widely used for caries prevention and enamel remineralization, concerns regarding long term fluoride overexposure including risks of dental and skeletal fluorosis, thyroid dysfunction, neurodevelopmental effects, and gastrointestinal disturbances have increased interest in natural oral hygiene solutions.
- Future research should focus on randomized clinical trials with larger sample sizes to further establish Miswak's role in orthodontic care and its potential as a mainstream adjunct to conventional oral hygiene practices.

Future studies could emphasize orthodontic or non-orthodontic patient education on the benefits of miswak and suggest developing tailored educational materials and programs. Future research could lead to the development and accessibility of more miswak-based oral care products, such as mouthwashes and dental gels, especially in culturally oriented regions.

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10. APPENDIX

Technical sheets of growth media (growth cultures):

- BD Trypticase
- HIMEDIA TYCSB
- Millipore MRS Agar



- **BD Trypticase**



INSTRUCTIONS FOR USE –
READY-TO-USE PLATED
MEDIA



PA-254053.07

Rev.: Mar 2013

BD™ Trypticase™ Soy Agar II with 5% Sheep Blood

INTENDED USE

BD Trypticase Soy Agar II with 5% Sheep Blood is a nutritious general purpose medium for the isolation and cultivation of nonfastidious and fastidious micro-organisms from clinical specimens and the detection of hemolytic reactions.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

The nutritional composition of **Trypticase Soy Agar (TSA)** has made it a popular medium, both unsupplemented and as a base for media containing blood.¹ **BD Trypticase Soy Agar II** is an improved version, providing clearer hemolytic zones than **Trypticase Soy Agar (TSA)** when supplemented with 5% sheep blood. It provides excellent growth and beta hemolysis of hemolytic streptococci and other organisms.²⁻⁴ It is suitable for performing the CAMP test (formation of a zone of complete, synergistic hemolysis at the junction of perpendicular streak inocula of *Staphylococcus aureus* and group B streptococci) for the presumptive identification of *Streptococcus agalactiae*, and for use with low concentration (0.04 unit) bacitracin discs (**BD Taxo™** A discs) for presumptive identification of group A streptococci (*S. pyogenes*).^{2,3,5,6}

BD Trypticase Soy Agar II with 5% Sheep Blood is not the appropriate medium for the primary isolation of fastidious anaerobes.

The combination of casein and soy peptones in the Trypticase Soy Agar (TSA II™) base render the medium highly nutritious by supplying organic nitrogen, particularly amino acids and larger-chained peptides. The sodium chloride maintains osmotic equilibrium. Proprietary growth factors improve the hemolytic reactions. Defibrinated sheep blood is the most widely used blood for enriching agar base media.¹

REAGENTS

BD Trypticase Soy Agar II with 5% Sheep Blood

Formula* Per Liter Purified Water

Pancreatic Digest of Casein	14.5 g
Papaic Digest of Soybean Meal	5.0
Sodium Chloride	5.0
Growth Factors	1.5
Agar	14.0
Defibrinated Sheep Blood	5%

pH 7.3 +/- 0.2

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only. ②

Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates in the dark at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

PA-254053.07

- 1 -

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate the inoculated plates at $35 \pm 2^\circ\text{C}$ in an aerobic atmosphere supplemented with carbon dioxide. Examine plates after 18 to 24 h for amount of growth, colony size and hemolytic reactions.

Strains	Growth Results
<i>Escherichia coli</i> ATCC™ 25922	Growth good to excellent; may be beta-hemolytic
<i>Enterococcus faecalis</i> ATCC 29212	Growth good to excellent
<i>Shigella flexneri</i> ATCC 12022	Growth good to excellent
<i>Staphylococcus aureus</i> ATCC 25923	Growth good to excellent; beta hemolysis
<i>Streptococcus agalactiae</i> ATCC 12386	Growth good to excellent; (weak) beta hemolysis
<i>Streptococcus pneumoniae</i> ATCC 6305	Growth good to excellent green-grey colonies; alpha hemolysis
<i>Streptococcus pyogenes</i> ATCC 19615	Growth good to excellent; beta hemolysis
Uninoculated	Red (blood color)

PROCEDURE

Materials Provided

BD Trypticase Soy Agar II with 5% Sheep Blood (90 mm **Stacker™** plates).
Microbiologically controlled.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

This is a universal isolation medium in aerobic bacteriology that can be used for all types of clinical specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Test Procedure

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. In order to improve detection of all pathogens contained in the specimen, it must also be streaked onto appropriate selective media.^{1,4}

Since many pathogens require carbon dioxide on primary isolation, plates should be incubated in an aerobic atmosphere containing approximately 3 to 10% CO₂. Incubate plates at 35-37°C for 18 to 24 h or longer, if necessary.

CAMP-Test: Isolates suspected to be *Streptococcus agalactiae* may be tested by the CAMP test for presumptive identification. The inoculum may be picked from a blood agar plate. Make a single streak of *Staphylococcus aureus* ATCC 33862 across the center of the plate. If a loop is used, do not use it parallel to the agar surface, since the streak will be too wide and the results will not be satisfactory. The streptococcal test strains are inoculated by making a single streak perpendicular to the *S. aureus* line coming as close as possible (2-3 mm), but not touching it. Several isolates may be tested on the same plate. Perpendicular streptococcal streaks should be 5-8 mm apart. It is recommended that *S. agalactiae* ATCC 12386 be included along with patient isolates to verify satisfactory performance and *S. pyogenes* ATCC 19615 as a negative control. The procedure should be practised with known cultures before using it to identify unknown isolates.

Note: Studies on the CAMP Test have shown that the reaction is most reliable early in the shelf life of some lots of the prepared plated medium.

For the CAMP test, incubate plates in an aerobic atmosphere at $35 \pm 2^\circ\text{C}$ for 18 to 24 h. Do not incubate anaerobically or in a CO₂ incubator since this may cause false-positive results with group A streptococci.^{2,3,5,6}

PA-254053.07

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- HIMEDIA TYCSB



Technical Data

Tryptone Yeast Extract Cystine w/Sucrose and w/o Bacitracin Agar M1975 (TYCSB)

Intended Use:

Recommended for selective isolation of *Streptococcus mutans*

Composition**

Ingredients	Gms / Litre
Tryptone	15.000
Yeast extract	5.000
Disodium hydrogen phosphate.7H ₂ O	1.000
Sodium bicarbonate	2.000
Sodium acetate.3H ₂ O	20.000
Sucrose	200.000
L-Cystine	0.200
Sodium sulfite	0.100
Sodium chloride	0.100
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 249.99 grams (equivalent weight of dehydrated medium per litre) in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile rehydrated contents of 1 vial of TYCSB supplement (FD321). Mix well and pour into sterile petri plates.

Principle And Interpretation

TYCSB Agar is devised by Gold et al. (1) as a selective medium for *Streptococcus mutans* with bacitracin and sucrose. *Streptococcus mutans* is facultatively anaerobic, Gram-positive coccus-shaped bacterium commonly found in the human oral cavity. It is the primary causative agent of dental cavities (2).

Conditions in the oral cavity are diverse and complex, frequently changing from one extreme to another. Thus, to survive in the oral cavity, *S.mutans* must tolerate rapidly harsh environmental fluctuations and exposure to various antimicrobial agents to survive (3).

Tryptone and yeast extract in the medium provide nutrients essential for the growth of Streptococci. Sodium sulphite, sodium acetate, disodium phosphate, and sodium bicarbonate are sources of ions that simulate metabolism.

Type of specimen

Clinical samples - Blood, Saliva Swab, Decaying tooth.(6)

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (4,5).

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

¹This medium is general purpose medium and may not support the growth of fastidious organisms.

Please refer disclaimer Overleaf.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel.

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction

Reaction of 24.99% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH

7.10-7.50

Cultural Response

Cultural characteristics observed in presence of 10% CO₂ + 90% H₂ with added TYCSB supplement (FD321), after an incubation at 35-37°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
<i>Streptococcus mutans</i> ATCC 50-100 25175		good-luxuriant	≥50%

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).

Reference

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Revision : 02 / 2019

Please refer disclaimer Overleaf.

Millipore MRS Agar

* Agar-Agar is equivalent to other different terms of agar.

** ISO 15214 states for the pH: In order that the pH-value does not fall below 5.6, the tolerance here is ± 0.1 instead of ± 0.2 as usual.

Preparation

Dissolve 68.2 g in 1 l of purified water. Heat in boiling water, and agitate frequently until completely dissolved. Autoclave 15 minutes at 121 °C or use 118 °C to achieve improved growth of *Bifidobacterium spp.*

The prepared medium is clear and brown.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by ISO 15214, inoculate the medium by using poured plate technique.

Incubate the inoculated plates inverted at 29-31 °C for 69-75 h.

According to ISO 15214, surface plating in combination with incubation under anaerobic or microaerobic conditions can be applied instead of the pour plating method. Anaerobic or microaerobic conditions can be achieved by using Merck Anaerocult® C or C mini in an anaerobic jar.

It is also possible to use a double-layer MRS.

Avoid desiccation of the agar during incubation so that the medium does not become too inhibitory by increasing the acetate concentration at the surface, which inhibits the growth of *Lactobacilli*.

Storage

Store at +2 °C to +8 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

According to Corry et al., self-prepared plates can be stored +2 °C to + 8 °C in the dark and protected against evaporation for up to 14 days.

Quality Control

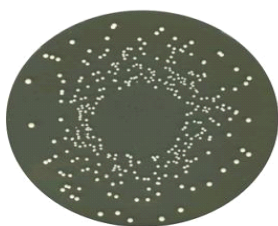
Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	<i>Lactobacillus acidophilus</i> ATCC® 4356	69-75 h at 29-31 °C aerobic	Previously validated batch of MRS agar	Quantitative	Recovery ≥ 70 %
	<i>Lactobacillus sakei</i> ATCC® 15521				
	<i>Lactococcus lactis</i> spp. <i>lactis</i> ATCC® 19435				
	<i>Pediococcus pentosaceus</i> ATCC® 33316				
	<i>Pediococcus pentosaceus</i> ATCC® 29358				
	<i>Bifidobacterium bifidum</i> ATCC® 11863	69-75 h at 29-31 °C anaerobic	-	Qualitative	Good growth

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Selectivity	<i>Escherichia coli</i> ATCC® 8739	69-75 h at 29-31 °C	-	Qualitative	Total inhibition
	<i>Escherichia coli</i> ATCC® 25922				
	<i>Bacillus cereus</i> ATCC® 11778				

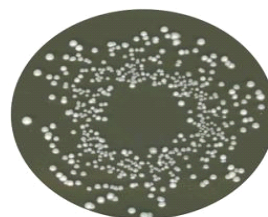
Please refer to the actual batch related Certificate of Analysis.

The performance test is in accordance with the current version of EN ISO 11133. A

recovery rate of 70 % is equivalent to a productivity value of 0.7.



Lactobacillus casei ATCC® 393



Bifidobacterium bifidum ATCC® 11863

Literature

APHA (2015): Compendium of Methods for the Microbiological Examination of Foods. 5th edition. American Public Health Association, Washington, D.C.

De Man J.D., Rogosa M., and Sharpe M.E. (1960): A Medium for the cultivation of *Lactobacilli*. J. Appl. Bact. **23**: 130-135.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of mesophilic lactic acid bacteria - Colony-count technique at 30 °C. ISO 15214:1998.

ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media. EN ISO 11133:2014.

Schillinger, U. and Holzapfel, W.H. (2012): Culture media for Lactic Acid Bacteria. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds.), pp 174-186. Royal Society of Chemistry, Cambridge, UK.

Ordering Information

Product	Cat. No.	Pack size
GranuCult™ MRS Agar (de MAN, ROGOSA and SHARPE) acc. ISO 15214	1.10660.0500	500 g
ReadyPlate™ MRS Medium ISO 15214	1.46717.0020	20 x 90 mm
ReadyPlate™ MRS Medium ISO 15214	1.46717.0100	100 x 90 mm
ReadyTube™ 200 MRS Medium ISO 15214	1.46364.0006	6 x 200 ml
Anaerobic jar	1.16387.0001	1 jar
Anaeroclip®	1.14226.0001	1 x 25
Anaerocult® C	1.16275.0001	1 x 10
Anaerocult® C mini	1.13682.0001	1 x 25
Plate basket	1.07040.0001	1 piece

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11. ETHICS COUNCIL REPORT

İSTANBUL MEDİPOL ÜNİVERSİTESİ
GİRİŞİMSSEL OLMAYAN KLİNİK ARAŞTIRMALAR
ETİK KURULU KARAR FORMU

Sayı : E-10840098-772.02-2952
Konu: Etik Kurulu Kararı

22/06/2021

BAŞVURU BİLGİLERİ	ARAŞTIRMANIN AÇIK ADI	“The effect of Salvadora Persica on the oral health of Orthodontic patients” “Salvadora Persica (Misvak)ın Ortodonti hastalarının ağız sağlığına etkisi”			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACI UNVANI/ADI/SOYADI	ZAKARİYA ABUHARFİL			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ UZMANLIK ALANI	Dt./Ortodonti Master Öğrenci			
	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ BULUNDUĞU MERKEZ	İstanbul			
	DESTEKLEYİCİ	-			
	ARAŞTIRMAYA KATILAN MERKEZLER	TEK MERKEZ ☒	ÇOK MERKEZLİ ☐	ULUSAL ☒	ULUSLARARASI ☐

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İSTANBUL MEDİPOL ÜNİVERSİTESİ
GİRİŞİMSEL OLMAYAN KLİNİK ARAŞTIRMALAR
ETİK KURULU KARAR FORMU

Değerlendirilen Belgeler	Belge Adı	Tarihi	Versiyon Numarası	Dili		
	ARAŞTIRMA PROTOKOLÜ/PLANI			Türkçe ☐	İngilizce ☐	Diğer ☐
	OLGU RAPOR FORMU			Türkçe ☐	İngilizce ☐	Diğer ☐
	BİLGİLENDİRİLMİŞ GÖNÜLLÜ OLUR FORMU			Türkçe ☐	İngilizce ☐	Diğer ☐
Karar Bilgileri	Karar No:702	Tarih: 17/06/2021				
	Yukarıda bilgileri verilen Girişimsel Olmayan Klinik Araştırmalar Etik Kurulu başvuru dosyası ile ilgili belgeler araştırmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş ve araştırmanın etik ve bilimsel yönden uygun olduğuna “oybirliği” ile karar verilmiştir.					

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BAŞKANIN UNVANI / ADI / SOYADI	Dr. Öğr. Üyesi Mahmut TOKAÇ

Unvanı/Adı/Soyadı	Uzmanlık Alanı	Kurumu	Cinsiyet		Araştırma ile ilişki		Katılım *		İmza
Dr. Öğr. Üyesi Mahmut TOKAÇ	Tıp Tarihi ve Etik	İstanbul Medipol Üniversitesi	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	Uygundur
Prof. Dr. Mete ÜNGÖR	Endodonti	İstanbul Medipol Üniversitesi	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	Uygundur
Doç. Dr. Mehmet Kemal ÖZDEMİR	Elektrik ve Elektronik	İstanbul Medipol Üniversitesi	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	Uygundur
Doç. Dr. İlknur KESKİN	Histoloji ve Embriyoloji	İstanbul Medipol Üniversitesi	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	Uygundur
Doç. Dr. Devrim TARAKCI	Fizyoterapi ve Rehabilitasyon	İstanbul Medipol Üniversitesi	E ☒	K ☐	E ☐	H ☒	E ☒	H ☐	Uygundur
Dr. Öğr. Üyesi Neziha HACIHASANOĞLU ÇAKMAK	Biyokimya	İstanbul Medipol Üniversitesi	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	Uygundur
Dr. Öğr. Üyesi Neriman İpek KIRMIZI	Tıbbi Farmakoloji	İstanbul Medipol Üniversitesi	E ☐	K ☒	E ☐	H ☒	E ☒	H ☐	Uygundur

* :Toplantıda Bulunma

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COVID-19 (Pandemi) nedeniyle etik kurulumuz sanal olarak toplanmış olup kurul üyelerimizden uygunluk kararı sanal ortamda alınmıştır. Araştırmacı tarafından talep edilirse, COVID-19 (Pandemi) sonrası ıslak imzalı karar formu ayrıca hazırlanabilir.

Girişimsel Olmayan Etik Kurulu Sekreteri
Bilge KAYA

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