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Evaluation of anti-plaque and anti-inflammatory efficacies of mouth rinse containing green tea and *Salvadora Persica L.* in the management of dental biofilm-induced gingivitis:

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Abstract:

The plaque accumulation is the main cause of gingivitis, Biofilm-induced gingivitis can be effectively treated by a combination of measures that include motivation and oral hygiene instruction (OHI), professional mechanical plaque elimination, in addition to the supplementary use of antibacterial and anti-inflammatory oral hygiene agents. Therapeutic mouthwash can improve oral hygiene, e.g., by reducing dental biofilm and gingivitis. Chlorhexidine (CHX) is the main mouthwash employed for chemical plaque reduction. CHX is associated with certain adverse effects, including extrinsic staining of teeth. The unwanted effects necessitate the development of new mouthwashes that are equally effective yet devoid of these issues. A mouth rinse containing Green Tea (GT) and *Salvadora Persica L.* (SP) was investigated for its use in the treatment of gingivitis, and they found that it is effective in reducing gingivitis.

Keywords: *Salvadora*; Green tea; Interleukin-6; Gingivitis; Dental plaque.

Introduction:

A bacterial infection is typically the cause of gingivitis. Unlike periodontitis, there is no attachment loss (Marchesan et al., 2020). Dental plaque-induced gingivitis is an inflammatory condition resulting from the interaction between dental plaque and the host's immune-inflammatory response at the affected site. The inflammation is confined to the gingiva and does not extend to the mucogingival junction (Murakami et al., 2018, Talib and Ahmed, 2018). In a clinical context, the gingival tissues exhibit certain characteristics such as swelling, redness, discomfort, a glossy appearance, and bleeding upon gentle probing (Trombelli et al., 2018). The utilization of biomarkers for the diagnosis of gingivitis is emerging as a significant concern in periodontal research (Abdullameer and Abdulkareem, 2023). Biomarkers are found in oral fluids, including saliva and gingival crevicular fluid (GCF) (Mahmood, 2024). Considering these facts, rapid chair-side assays utilizing biomarkers present in biological fluids have been established for the diagnosis of periodontal disease. These assessments, referred to as point-of-care (POC) diagnostics, streamline diagnosis and

enhance prognosis (Mahmood and Abbas, 2023, Mohammed et al., 2024). Interleukin-6 (IL-6) is a pleiotropic cytokine that exhibits both pro-inflammatory and anti-inflammatory activities (Nanakaly, 2016). The cytokine interleukin-6 (IL-6) exerts various physiological effects, including bone resorption, activation and differentiation of macrophages and T cells, growth and differentiation of B cells, stimulation of hematopoiesis, and differentiation of osteoclasts (Naruishi and Nagata, 2018). IL-6 has been found in elevated levels in gingival crevicular fluid (GCF) and saliva. It has been associated with disease extent/severity and has been shown to decrease with successful therapy (Salminen et al., 2014, Muhammed, 2022). The mouthwash containing chlorhexidine (CHX) has recently become the most powerful chemotherapeutic drug and the standard for decreasing *S. mutans* and oral biofilm (S et al., 2019). The (CHX) mouthwash is widely used as a reference standard, but CHX had been associated with extrinsic staining and increased calculus formation (Slot et al., 2014). Due to these adverse effects, there is an important reason to create alternative mouthwashes that are equally effective yet devoid of these issues (Pradeep et al., 2016). Therefore, the mouthwash containing a blend of aqueous extracts from *Salvadora persica* L. and green tea serves as a substitute for CHX, exhibiting minimal side effects (Mehta et al., 2013).

Dental biofilm-induced gingivitis:

A bacterial infection is typically the cause of gingivitis. The junctional epithelium does not migrate because there is no attachment loss, unlike periodontitis, the disease only affects the gingival epithelium and connective soft tissues (Marchesan et al., 2020, misanjas et al., 2024). Gingivitis is the most prevalent among all periodontal disorders. Gingivitis can present in various forms based on clinical presentation, duration of infection, severity, and underlying causes. The predominant form of gingivitis is attributed to dental plaque accumulation. Clinically, gingivitis exhibits signs of swelling, redness, soreness, a glossy appearance, and bleeding when gently probing. Gingivitis rarely causes spontaneous bleeding and is typically devoid of discomfort, resulting in many patients being unaware of the condition and neglecting to seek medical assistance (Trombelli et al., 2018). Gingivitis may be categorized as either localized or generalized. Localized gingivitis is diagnosed when a patient presents with bleeding on probing (BOP) between 10% and 30%. On the other hand, generalized gingivitis is diagnosed when a patient presents with a BOP of more than 30% (Chapple et al., 2018).

Dental plaque:

Dental plaque is a complex biofilm that covers tooth enamel and other hard oral tissues. The application of polymerase chain reaction (PCR) methodologies for microbial detection has shown the presence of more than 500 microbial species as natural residents of dental plaque. The biofilm primarily consists of bacteria, but it may also include yeasts, protozoa, Archaea, and viruses, all completely enclosed within a self-generated matrix of extracellular polymeric substances (EPS) (Rosan and Lamont, 2000). The bacteria and the secreted matrix of EPS are the two important components of a biofilm. The EPS promotes bacterial attachment, encourages biofilm growth, and maintains the overall integrity of the biofilm (Lang et al., 2015). Clinically, dental plaque is a persistent, tenacious, yellowish-grey layer that forms on the hard surfaces of the oral cavity, including both permanent and removable dental restorations (Newman et al., 2023). The plaque formation process can be categorized into multiple stages shown in (Figure 1) (Abdulkareem et al., 2023).

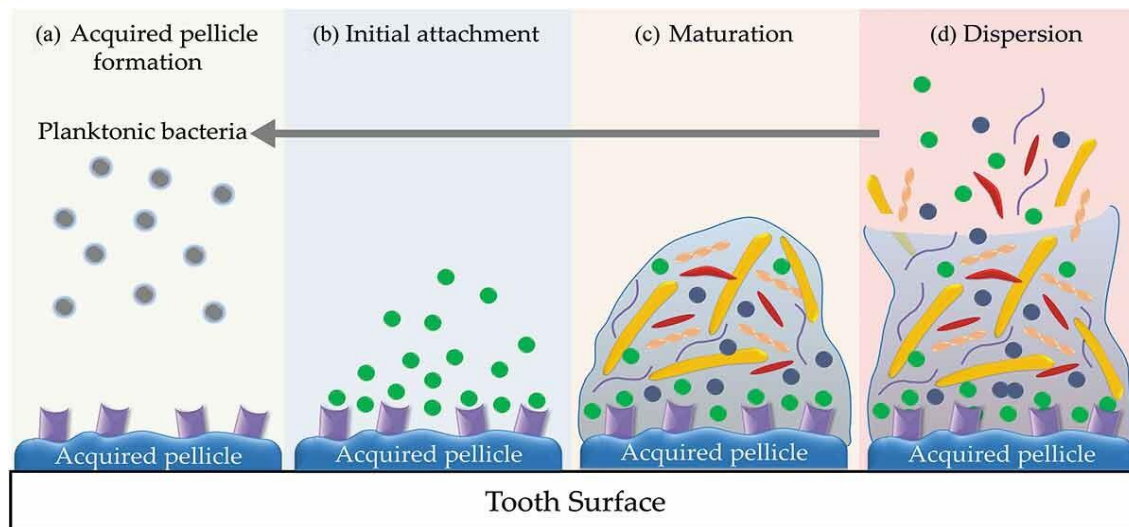


Figure 1: Formation of dental plaque biofilm a. acquired pellicle formation; b. initial attachment; c. Colonization and Plaque Maturation; D. Dispersion of biofilm cells (Abdulkareem et al., 2023).

Mechanical plaque control:

The most commonly used method for plaque control is mechanical disorganization or removal of the plaque. Mechanical plaque control involves regularly removing microbial deposits that build up on tooth surfaces, gingival margins, and other oral hard surfaces to prevent their recurrence through self-care or professional treatment, which is crucial for maintaining periodontal health (Akram, 2015). The success of a self-care mechanical plaque management strategy is dependent upon several factors, including physical dexterity, motivation, knowledge, the choice of oral hygiene instruments, and the provision of oral hygiene instructions (Axelsson et al., 2002, Ayoub and Abdulbaqi, 2024).

Chemical plaque control:

Chemical plaque control is the use of chemical substances to control the formation and growth of dental plaque. These agents can be applied using different methods, including dentifrices, mouth rinses, and gels (Axelsson et al., 2002). According to several studies, The CHX was considered the most important agent used in the treatment of periodontal disease. The use of chemical plaque regulation was employed as a supplementary method, rather than a substitute for mechanical means. Further, Enhances the efficacy of plaque management regimens by including anti-plaque chemicals alongside mechanical plaque control (Vyas et al., 2021).

Chlorhexidine:

The CHX mouthwash has recently become the most powerful chemotherapeutic drug and the standard for decreasing *S. mutans* and oral biofilm (Shrimathi et al., 2019). CHX products are used in dentistry and come in multiple forms, including mouthwash, gels, chips, and varnishes (Walsh et al., 2015). As a mouth rinse the intriguing characteristic of CHX is its substantivity, which refers to its gradual release into the oral cavity following its adsorption. This extended antibacterial activity that lasts up to 12 hours is why CHX has the highest anti-plaque effectiveness, which has not been overtaken by any other mouth rinses (Tomás et al., 2010). The CHX exhibits a wide range of antibacterial properties, effectively targeting various types of bacteria, including gram-positive and gram-negative organisms, facultative anaerobes, aerobes, and yeasts (Lee et al., 2016). The CHX is

known as the preferred method for chemical plaque management and has proven to be an efficient anti-plaque and anti-inflammatory agent (Padol et al., 2022).

Limitation of chlorhexidine mouthwash:

The Side effects of CHX may arise even at therapeutically small dosages of chlorhexidine (CHX) mouthwash (0.06% to 0.2%) (James et al., 2017). Multiple self-reported adverse effects were seen, including changed taste perception, oral and tongue numbness, discomfort in the mouth and tongue, xerostomia, and observed discoloration. While the 0.12% and 0.2% concentrations showed a higher incidence of "loss of taste" and "numb feeling" compared to the 0.06% concentration, no cases of serious adverse effects like erosion and ulceration of the oral mucosa were recorded (Deus and Ouanounou, 2022). Long-term use may lead to the formation of calculus and staining on the teeth. Less common adverse effects encompass parotid gland enlargement, oral paraesthesia, glossodynia, hypersensitivity, burning sensations, and desquamation of the oral mucosa. However, teeth discoloration continues to be the principal adverse effect that prevents people from using CHX (Brookes et al., 2020).

Camellia sinensis (L.):

Tea, sourced from the leaf and bud of the *Camellia sinensis* plant, is widely consumed worldwide as the second most popular beverage (Zohora and Arefin, 2022). Tea, originating in China, has been popular worldwide for the last 2000 years. Tea holds significant economic and social importance, being used regularly by many individuals both as a common beverage and as a medicinal remedy for many ailments (Abdulbaqi et al., 2016a). The major components of tea are polyphenols, specifically monomeric flavanols, referred to as catechins (Nugala et al., 2012). Moreover, research has shown that catechins may have a substantial impact on the prevention of cancer formation (Mao et al., 2019), lowering of plasma cholesterol levels, protection from cardiovascular diseases (Xing et al., 2019), enhanced loss of body fat (Zhang et al., 2012), protection against infectious diseases (Reygaert, 2018), protection against neurodegenerative diseases (Pervin et al., 2019) and also improving fertility (Rahman et al., 2018).

Types of Camellia sinensis (L.) Kuntze:

Camellia sinensis is a member of the tea family, Theaceae, and is found in China, Sri Lanka, Japan, and India. *Camellia sinensis* var. *assamica* is recognized as one of the primary varieties of green tea plants due to its economic importance. The tree is known for its rapid growth and tall stature, with large leaves as a distinguishing feature. Tea prepared from the leaves of this plant is known as Assam tea because it's associated with India's Assam district. On the other hand, *Camellia sinensis* var. *sinensis* is a bush that grows at a slower pace and has delicate leaves, making it capable of withstanding colder temperatures. This particular variety, also referred to as the China tea plant, is extensively cultivated in China, Japan, and Taiwan (Habtemariam, 2019).

Antiplaque activity of green tea:

A wide range of studies have been conducted to examine the effectiveness of green tea in inhibiting plaque development. A single-blinded cross-over clinical trial was performed on 30 individuals aged 18 to 25 to assess the efficacy of a 0.25% green tea catechins mouth rinse versus a 0.12% chlorhexidine gluconate mouth rinse. The study demonstrates that green tea catechin

mouthwash is as effective as chlorhexidine gluconate in reducing plaque after 7 days of administration. Additionally, green tea catechin mouthwash, which has a better flavor and has no reported negative effects, can be regularly used as a substitute for chlorhexidine gluconate as an anti-plaque agent (Kaur et al., 2014). Another study was conducted involving 60 healthy children aged 9-14 years. This study followed a randomized blinded controlled trial design. The participants were assigned to three groups in a random manner and instructed to rinse twice daily with either 0.5% C. sinensis extract mouth rinse, 0.2% chlorhexidine gluconate, or 0.05% Sodium fluoride over a period of two weeks with 20 subjects per group. The assessment of plaque formation and gingival condition was conducted using the plaque index and gingival index. The study results indicate no statistically significant difference in the efficacy of green tea mouthwash compared to chlorhexidine gluconate during a 14-day duration (Hambire et al., 2015).

Antimicrobial activity of green tea:

Research has shown that green tea extracts have antibacterial characteristics, specifically targeting many types of oral bacteria, including both gram-positive and gram-negative strains such as *E. coli*, *Salmonella spp.*, *Staphylococcus aureus*, and *Enterococcus spp.* (Alghamdi, 2023). Green tea consists of polyphenols, which are made of catechins. Epigallocatechin-3-gallate (EGCG) is the most prevalent catechin and a significant contributor to this effect. EGCG demonstrates a more pronounced effect on gram-positive bacteria in comparison to gram-negative bacteria. Studies have demonstrated that higher concentrations of EGCG lead to a reduction in bacterial populations (You, 1993). Investigations conducted in the last two decades has discovered that specific polyphenolic catechins present in green tea, such as (-)-epigallocatechin gallate (EGCG) and (-)-epicatechin gallate (ECG), possess the capacity to moderately hinder the growth of different types of bacteria, including both Gram-positive and Gram-negative bacteria. These substances demonstrate potential for reducing common oral infections, including dental caries and periodontal disease. The antimicrobial activity of green tea is attributed mainly to its catechins, specifically EGC, EGCG, and ECG, which are the essential antibacterial compounds (Taylor, 2020).

Anti-inflammatory effects of green tea:

Multiple investigations have demonstrated that green tea extract (GTE) possesses anti-inflammatory properties. GTE and its main component, epigallocatechin-3-gallate (EGCG), have shown effectiveness in decreasing inflammation produced by lipopolysaccharide (LPS) (Azambuja et al., 2022). Furthermore, research has shown that extracts from Green tea have the ability to reduce the levels of pro-inflammatory cytokines and increase the levels of anti-inflammatory markers. This suggests that Green tea may have a beneficial effect on inflammation (Bagheri et al., 2020). Green tea and its bioactive components have been found to offer numerous health benefits and may help prevent certain diseases. These benefits encompass a range of effects, including reducing inflammation, combating oxidative stress, preventing cancer, and regulating metabolism. Green tea's various modes of action make it an interesting choice for improving inflammatory conditions (Sun et al., 2022).

Salvadora persica L:

Salvadora persica Linn., also known as miswak or toothbrush, is a member of the Salvadoraceae family (Ronse De Craene and Wanntorp, 2009). *Salvadora persica* L. is a perennial shrub that reaches a height of 4-6 meters. It features a compact trunk, white bark, and glossy green leaves. *Salvadora persica* L. is known for its strong efficacy in treating dental issues. The tree is commonly known as the Meswak tree, as its roots and twigs have been utilized for dental hygiene purposes since ancient times. It is a widely utilized medicinal plant for maintaining dental hygiene throughout the global Muslim community (Sher et al., 2010).

Antiplaque activity of *Salvadora persica* L:

The Correct utilization of chewing sticks, such as *Salvadora persica* L., is essential for enhancing oral hygiene and managing supragingival dental plaque. They can be equally effective in the removal of dental plaque as conventional toothbrushes (Goyal et al., 2009). According to an observational descriptive cross-sectional research including 528 patients, *S. persica* miswak was shown to be more beneficial than toothbrushes alone in decreasing gingivitis (Shetty et al., 2010). A clinical trial has examined the antiplaque effects of *Salvadora persica* L. mouth rinse and compared it to 0.2% chlorhexidine mouth rinse. The study included the random allocation of mouth rinses to all participants, who were instructed to rinse with the assigned mouth rinse twice a day in addition to regular tooth brushing. The study's findings indicated that there was no notable distinction observed between the use of *Salvadora persica* L. and 0.2% chlorhexidine as a supplement to tooth brushing (Rahmani and Radvar, 2005). In a study conducted by Abdulbaqi et al. (2016), it was demonstrated that the combination of green tea and *S. persica* root stick exhibited a synergistic antiplaque activity against primary colonizers of dental plaque in vitro. This study highlights the importance of investigating the combined bioactivity of *S. persica* with other medicinal plants, particularly those traditionally used in oral care. Such research has the potential to yield innovative bioactive polyherbal formulations (Abdulbaqi et al., 2016b).

Antimicrobial properties of *Salvadora persica* L:

Examining miswak extracts' antibacterial properties against a variety of human infections has been the subject of a significant amount of research. Multiple studies have demonstrated the notable antimicrobial properties of miswak (*Salvadora persica*) against both aerobic and anaerobic bacteria. Al-Bayati and Sulaiman conducted a study to examine the antimicrobial effects of aqueous and methanol extracts of *Salvadora persica* using disc diffusion and micro-well dilution assays. The research employed seven different oral microorganisms to assess the effectiveness of the extracts. These microorganisms included *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus faecalis*, *Streptococcus pyogenes*, *Lactobacillus acidophilus*, *Pseudomonas aeruginosa*, and *Candida albicans*. The antimicrobial assays revealed that the aqueous extract of *Salvadora persica* exhibited activity against all tested pathogens. Furthermore, the aqueous extract had stronger inhibitory efficacy than the methanol extract. It is noteworthy that the methanol extract had little efficacy against *Lactobacillus acidophilus* and *Pseudomonas aeruginosa* (Al-Bayati and Sulaiman, 2008, Haque and Alsareii, 2015). SP has a powerful antibacterial action on oral infections, particularly Gram-positive and Gram-negative bacteria (Abd Hesham et al., 2016). Research indicates that Sp extracts can

eradicate *S. mitis*, *S. sanguinis*, and *A. viscosus* in mixed solutions (Abdulbaqi et al., 2016a). The antibacterial properties of *S. persica* arise from its distinct chemical ingredients. Benzyl isothiocyanate (BITC) is an important compound obtained from the roots of *S. persica*. It has been demonstrated to be particularly effective against Gram-negative bacteria, including *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. BITC exhibits lipophilic and electrophilic characteristics. Some have postulated that it may influence the bacterial membrane potential by penetrating the outer bacterial membrane and disrupting bacterial redox processes (Sofrata et al., 2011).

Anti-inflammatory and Anti-oxidant effects of *Salvadora persica* L:

Salvadora persica L. demonstrates notable anti-inflammatory properties, supported by various studies. Research indicates that SP demonstrates antioxidant and anti-inflammatory properties, suggesting its potential for therapeutic applications. An analysis employing LC-ESI-MS/MS was performed on the SPE, indicating the presence of multiple bioactive compounds. The most prevalent chemicals were 3-formylindole, isosakuranetin-7-O-neohesperidoside, hydroxybenzoic acid, rhoifolin, spermidine, pantothenate, diosmin, and acacetin-7-O-rutinoside. The anti-inflammatory and antioxidant properties of *Salvadora persica* may be attributed to these components (Binsuwaidan et al., 2023). Previous investigations have demonstrated that SP possesses antioxidant properties by effectively neutralizing various free radicals. The existence of powerful antioxidant enzymes and chemicals, including flavonoids like luteolin, quercetin, and apigenin, as well as p-coumaric, ferulic, sinapic, and cinnamic acids, and furan derivatives, enhances this impact. Two prominent tocopherols (-tocopherol and -tocopherol) were present in the SP seed. Both compounds demonstrated antioxidant properties equivalent to those of vitamin E, highlighting their significance for human health (Mekhemar et al., 2021). Furthermore, SP has been experimentally demonstrated to possess significant anti-inflammatory effects, thereby confirming its antioxidant mechanisms (Nordin et al., 2020). It suppresses pro-inflammatory cytokines such as TNF- α , IFN, IL-1 β , IL-6, and IL-8, and modifies the isoforms of nitric oxide synthase (NOS) (Lebda et al., 2018), with a simultaneous release of α -Amylase enzyme promoting anti-inflammatory and antioxidative actions at the exact location of inflammation (Mohamed et al., 2014).

Conclusion:

1-Rinsing with a mixture of aqueous extracts of Gt and Sp, at concentrations of 0.25 mg/ml and 7.82 mg/ml respectively, can significantly reduce plaque buildup when performed twice daily for one month.

2-The mouthwash containing GT/SP demonstrated an anti-inflammatory effect, suggesting its potential for immune system modulation. These results suggest that GT/SP mouthwash could be an acceptable replacement for chlorhexidine (CHX) for treating gingivitis

الخلاصة:

يعد تراكم طبقة اللائحة الجرثومية السبب الرئيسي لالتهاب اللثة، ويمكن علاج التهاب اللثة الناجم عن طبقة اللائحة الجرثومية بشكل فعال من خلال مجموعة من التدابير التي تشمل التحفيز وتعليمات نظافة الفم والقضاء الميكانيكي على اللائحة الجرثومية، بالإضافة إلى الاستخدام التكميلي لعوامل نظافة الفم المضادة للبكتيريا والالتهابات. يمكن لغسول الفم العلاجي الكلور هكسدين تحسين نظافة الفم، على سبيل المثال، عن طريق تقليل طبقة اللائحة الجرثومية للأسنان والتهاب اللثة. يعد الكلور هيكسيدين رئيسي لتقليل طبقة اللائحة الجرثومية. يرتبط الكلور هيكسيدين ببعض الآثار الضارة، بما في ذلك تصبغ الأسنان الخارجي. تتطلب الآثار غير المرغوب فيها تطوير غسولات فم جديدة بنفس الفعالية ولكنها خالية من هذه المشكلات. تم لاستخدامه في علاج التهاب اللثة، ووجدوا أنه المسواك وشاي الأخضر فعال في تقليل التهاب اللثة.

الكلمات الرئيسية: المسواك؛ الشاي الأخضر؛ إنترلوكين 6؛ التهاب اللثة؛ لوحة الأسنان.

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Conflicts of Interest Statement.....

Manuscript title:

Evaluation of anti-plaque and anti-inflammatory efficacies of mouth rinse containing green tea and *Salvadora Persica L.* in the management of dental biofilm-induced gingivitis

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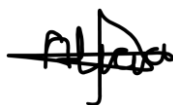
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