Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(4), 911-916 | ISSN:2251-6727

Cariogenic Biofilm Assessment of Herbal Extract Loaded Oral Patch

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(Received: 16 May 2025 Revised: 20 June 2025 Accepted: 12 July 2025)

KEYWORDS
Cariogenic biofilm, oral patch, oral health, herbal extract, colonizers.

ABSTRACT:
Introduction: Dental caries is a condition in which biofilm production destroys the mineralized tooth. Dental caries cannot be produced only by the presence of bacteria because the host diet is necessary for the formation of cariogenic biofilms. This study aims at using herbal extracts to provide insights into the effectiveness of the herbal extract-loaded oral patch in controlling cariogenic biofilm formation, reducing bacterial viability, and potentially preventing dental caries.

Objectives: This study aims at using herbal extracts to understand the effectiveness of the herbal extract-loaded oral patch in controlling cariogenic biofilm formation, reducing bacterial viability, and potentially preventing dental caries .

Methods: 6% of alginate and 3% of cellulose was dissolved in 100ml distilled water. 15ml of tulsi extract was added and this was mixed for 3 hours.The hydrogel was thus transferred to petri dish to form a membrane and kept at -4 degree celsius for 12 hours.Biofilm assessment with microorganisms . The fabricated membrane with and without extract is incubated with microbes (E.coli, E.faecalis, S.aureus, S.mutans) to form cariogenic biofilm. The biofilm assessment using SEM shows the destruction of bacterial membranes.

Results: The results showed that the O.tenuiflorum extract loaded oral patch showed reduction in cariogenic biofilm. According to the study the biofilm assessment showed destruction of microbes such as E.coli, E.faecalis, S.aureus, S.mutan. Observed Porous flexible and sheet-like morphology under Scanning Electron Microscope.

Conclusions: As per the study it is found that O.tenuiflorum extract loaded membrane showed reduction in the cariogenic biofilm formation.

1. Introduction

Dental caries is a condition in which biofilm production destroys the mineralized tooth. Dental caries cannot be produced only by the presence of bacteria because the host diet is necessary for the formation of cariogenic biofilms (1). Microbial interactions in the mouth are initiated by early colonizers that may cling to the pellicle-coated tooth surface quickly and then adhere with other bacteria (2). The physical and metabolic interactions among the various species throughout this time frame shape the early biofilm community.(1)(2)

Dental caries is caused by acidogenic and aciduric Gram-positive bacteria, including *actinomycetes, lactobacilli, and Mutans streptococci*. Periodontal diseases have been linked to anaerobic Gram-negative bacteria, including *Porphyromonas gingivalis, Actinobacillus, Prevotella, and Fusobacterium* (3). The prevalence of oral disease, the increase in bacterial antibiotic resistance, the side effects of some antibacterial agents currently used in dentistry, and the financial limitations in developing countries necessitate the development of safe, effective, and reasonably priced alternative preventive and treatment options (4). Many commercially available medications can alter the oral microbiota and cause undesirable side effects such diarrhea, vomiting, and tooth discolouration. Therefore, in the continuous search for alternatives, natural phytochemicals that are derived from plants and utilized as traditional remedies are seen as good equivalents (5).

Many studies have documented the beneficial use of natural remedies and traditional botanicals in the treatment of oral problems (6). Several medications derived from plants that are utilized as infection-treating agents in conventional medical systems are included in pharmacopeias (7). The efficacy of several of these drugs in treating oral microbial illnesses has recently been investigated. The extensive antibacterial qualities of therapeutic plants and plant derivatives, like essential oils, have been reviewed in the past. There have been reports of antibacterial and/or anti biofilm action in extracts from a variety of plants (8). The findings are mostly linked to the existence of secondary compounds, which are crucial for microbial pathogen resistance and defense against toxins and free radicals. These chemical compounds include flavonoids, phenolic acids, and tannins (9)

This study aims at using herbal extracts to understand the effectiveness of the herbal extract-loaded oral patch in controlling cariogenic biofilm formation, reducing bacterial viability, and potentially preventing dental caries (10).

2. Objectives

The primary objective of this study was to evaluate the effectiveness of a herbal extract-loaded oral patch, specifically incorporating *Ocimum tenuiflorum* (tulsi), in inhibiting cariogenic biofilm formation and reducing bacterial viability. The study aimed to explore a natural, biocompatible alternative to conventional antimicrobial agents in managing oral biofilms that contribute to dental caries. Through the formulation of a hydrogel-based oral patch using natural polymers such as alginate and cellulose, the study intended to assess its anti-biofilm properties against clinically relevant oral pathogens including *Streptococcus mutans, Escherichia coli, Enterococcus faecalis,* and *Staphylococcus aureus.* The objectives also included morphological analysis using Scanning Electron Microscopy (SEM) to visually confirm microbial inhibition, biofilm disruption, and structural characteristics of the patch, thereby validating its potential as an effective and safe approach for oral healthcare and dental caries prevention.

3. Methods

The herbal oral patch was created using natural polymers combined with a herbal extract. To prepare the hydrogel base, 6% alginate and 3% cellulose were dissolved in 100 mL of distilled water. These biopolymers were chosen for their compatibility with the body, ability to form films, and proven safety in both medical and dental uses. Once the base was ready, 5 mL of tulsi extract was added, and the mixture was stirred continuously for three hours to ensure the extract was evenly distributed.

Fabrication of the Herbal Extract-Infused Patch: After thorough mixing, the hydrogel was poured into a petri dish to create a membrane. This setup was then stored at 4°C for 12 hours, allowing the material to solidify and form a film with consistent thickness and composition. The finalized patch was then used for further analysis and testing.

Biofilm Assessment with Microorganisms: To evaluate the anti-biofilm efficacy of the developed nanofiber patches, in vitro assays were conducted using a panel of clinically relevant oral pathogens, including Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, and Streptococcus mutans. Both experimental membranes loaded with Ocimum tenuiflorum extract and control membranes devoid of the extract were subjected to identical conditions to facilitate comparative analysis.

The primary aim of this assessment was to determine the capacity of the O. tenuiflorum-incorporated membranes to inhibit bacterial adhesion, disrupt established biofilms, and induce morphological damage to microbial cells. Scanning Electron Microscopy (SEM) served as a pivotal tool for characterizing the surface interactions between the membranes and the bacterial cells. This technique enabled high-resolution visualization of biofilm architecture and the extent of bacterial membrane disruption following exposure to the test materials. Prior to SEM analysis, the nanofiber patches were prepared by sputter-coating the sample surfaces with a thin conductive layer of gold and palladium for 120 seconds using a Quorum SC7620 sputter coater. This coating was essential to minimize charging and enhance image clarity during electron beam exposure. Morphological examinations were subsequently performed using an EVO 40 LS 10 Zeiss SEM, which facilitated detailed observation of nanofiber surface topography and microbial colonization patterns.

4. Results

The results suggest that the O.tenuiflorum extract loaded patch exhibits promising anti-biofilm properties, making it a viable option for oral health applications, particularly

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www.jchr.org
JCHR (2025) 15(4), 911-916 | ISSN:2251-6727

in the prevention of dental caries. The biofilm evaluation indicated that the patch infused with O. tenuiflorum extract effectively minimized the formation of cariogenic biofilms. These biofilms, primarily formed by bacteria like Streptococcus mutans, Escherichia coli, Enterococcus faecalis, and Staphylococcus aureus, are key factors in the development of dental plaque and, ultimately, tooth decay. The findings of the study, based on both qualitative and quantitative assessments, confirmed a decrease in microbial proliferation and biofilm formation in the presence of the O. tenuiflorum extract.

Scanning Electron Microscope (SEM) images confirmed the destruction and inhibition of bacterial membranes, suggesting that the patch disrupts bacterial viability and biofilm structural integrity. The SEM analysis further revealed a porous, flexible, and sheet-like morphology of the patch, which enhances its potential adherence and function in the oral environment.

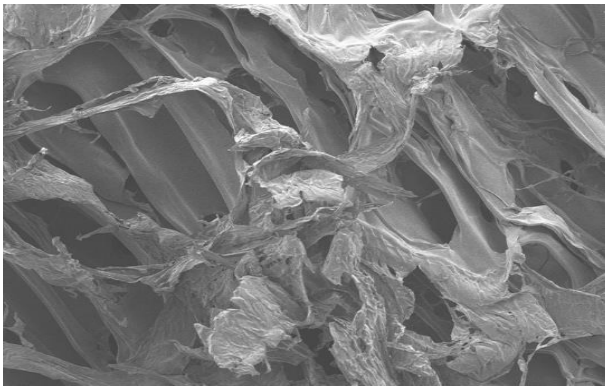

Figure 1 : SEM analysis showing porous morphology

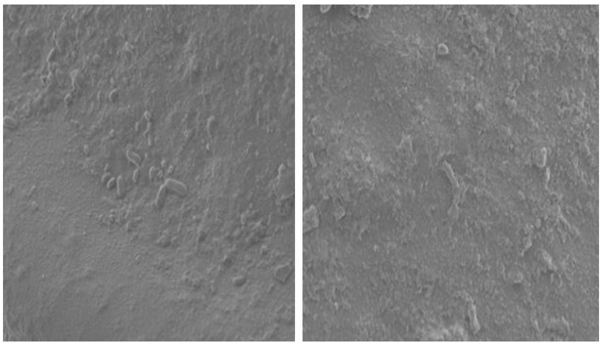

Figure 2: SEM (Scanning Electron Microscope) image, taken at a magnification of 3500x, shows the surface morphology of cariogenic biofilm analysis

The surface appeared to be uniformly smooth. The texture resembles a patch laden with herbal extract and represents a treated or uncolonized area. There are a few, dispersed, and non-clumped bacterial cells that are rod-shaped and coccoid. The bacteria seem loosely adhered or partially incorporated, suggesting that the surface may contain antimicrobial or anti-adhesive qualities. Extracellular polymeric material (EPS), which is normally present in mature biofilms, is not visible. This absence demonstrates that the formation of a dense bacterial community was successfully suppressed by biofilm growth.

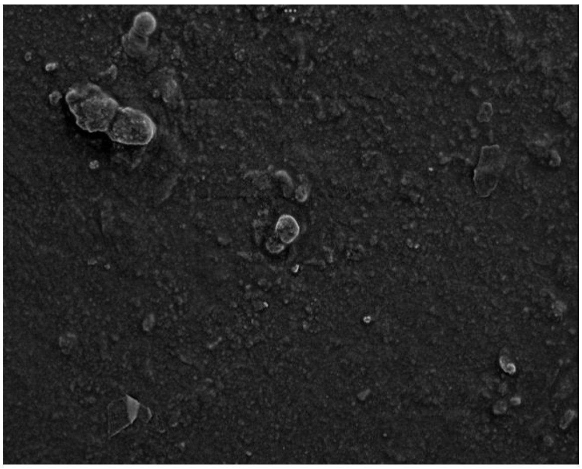

Figure 3 : This SEM (Scanning Electron Microscope) image, taken at 4500x magnification, provides surface morphology on an untreated or control surface.

Several distinct bacterial cells are visible, predominantly in coccoid (spherical) and rod-shaped forms. These morphologies are typical of common oral pathogens like Streptococcus mutans and Lactobacilli species. The clusters of bacteria and their attachment to the surface are strongly indicative of early-to-mature biofilm development.

The surface appears rough and uneven, and it is densely populated with small granules and microorganisms. The presence of extracellular polymeric substances (EPS) or debris-like material suggests active biofilm matrix formation, which protects bacteria from antimicrobial agents. The image demonstrates active microbial colonization, with multiple bacterial colonies forming microcolonies.

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5. Discussion

According to the study's findings, an oral patch loaded with Ocimum tenuiflorum (tulsi) extract effectively inhibits the formation of cariogenic biofilms, especially when it comes to common oral pathogens like Escherichia coli, Enterococcus faecalis, Staphylococcus aureus, and Streptococcus mutans. SEM analysis showed distinct morphological alterations in the biofilm structure and bacterial colonies, signifying bacterial lysis and membrane degradation (11). This antimicrobial action can be attributed to the well-documented phytoconstituents in tulsi, including eugenol, ursolic acid, and carvacrol, which are known to disrupt microbial membranes and inhibit enzyme activities (12).

According to a study by Sivasankari and Arulmozhi et al., herbal dental films containing neem and clove extracts showed a similar decrease in bacterial viability and biofilm development. They attributed this result to the phytochemicals' synergistic antibacterial qualities (13). Similarly, Saha et al developed alginate-based oral films and observed that films loaded with plant extracts showed good flexibility and antimicrobial activity (14) . These results closely resemble the current investigation, especially in terms of antibacterial effectiveness. The selection of the herbal extract, however, differs significantly; other studies have used mixtures of herbs, but the current study only uses O. tenuiflorum (tulsi), which is well-known for its immunomodulatory and broad-spectrum antibacterial effect (Mondal et al., 2009). This single-extract method streamlines formulation and might lessen efficacy variability, but it might also restrict the synergistic benefits that multi-extract systems provide (15).

While many earlier studies relied primarily on agar diffusion or spectrophotometric assays to assess microbial activity, the current study utilizes SEM, providing direct visual evidence of biofilm disruption and morphological changes in bacterial cells (16) This is a more robust and reliable method of analysis, offering microstructural insights that quantitative methods alone may miss. A similar study done by Sara et al observed that, Green tea can be used for dental caries prevention as both alcoholic and aqueous extracts have antibiofilm activity against the cariogenic bacteria Streptococcus mutans (17). Thus, these extracts can be used for preparation of antibiofilm dental formulas to be applied for therapeutic purposes or in oral hygiene practice.

The observed porous and sheet-like morphology of the patch enhances surface area for interaction with the oral mucosa, facilitating better mechanical integration in the oral cavity. This morphological characteristic, confirmed by SEM, was not described in as much detail in many previous studies (18). Moreover, while studies like those by Sharma et al. emphasize antioxidant properties of herbal patches, the current study focuses more on biofilm inhibition and mechanical robustness, a practical approach that may have greater clinical relevance (19)(21).

However, sustained release kinetics and long-term stability, two crucial factors for practical implementation are not assessed in this work. Sultana et al.'s study, on the other hand, looked at the time-based drug release patterns from herbal patches and found that adding release-modifying agents can increase efficacy over a period of 8 to 12 hours (20)(22). This represents an area for further optimization in the O.tenuiflorum patch formulation. Similarly, patient compliance and taste masking are also critical in practical deployment .

6. Conclusion

The study successfully demonstrated that Ocimum tenuiflorum extract-loaded oral patch exhibits significant potential as an effective, natural alternative for managing oral biofilms and preventing dental caries. The patch showed strong antimicrobial activity against key cariogenic bacteria, including Streptococcus mutans, as confirmed by biofilm inhibition and SEM analysis. Compared to similar herbal-based oral care systems, this formulation stands out due to its single-herb approach, strong antimicrobial efficacy, and robust structural integrity. Overall, this oral patch represents a promising, eco-friendly, and biocompatible solution for oral healthcare, with potential applications in preventing plaque formation and reducing bacterial load in the oral cavity. Further in vivo studies and clinical trials are required to establish its long-term effectiveness and patient compliance.

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