

Design and optimization of a synergistic herbal mucoadhesive film for the treatment of oral pathogens

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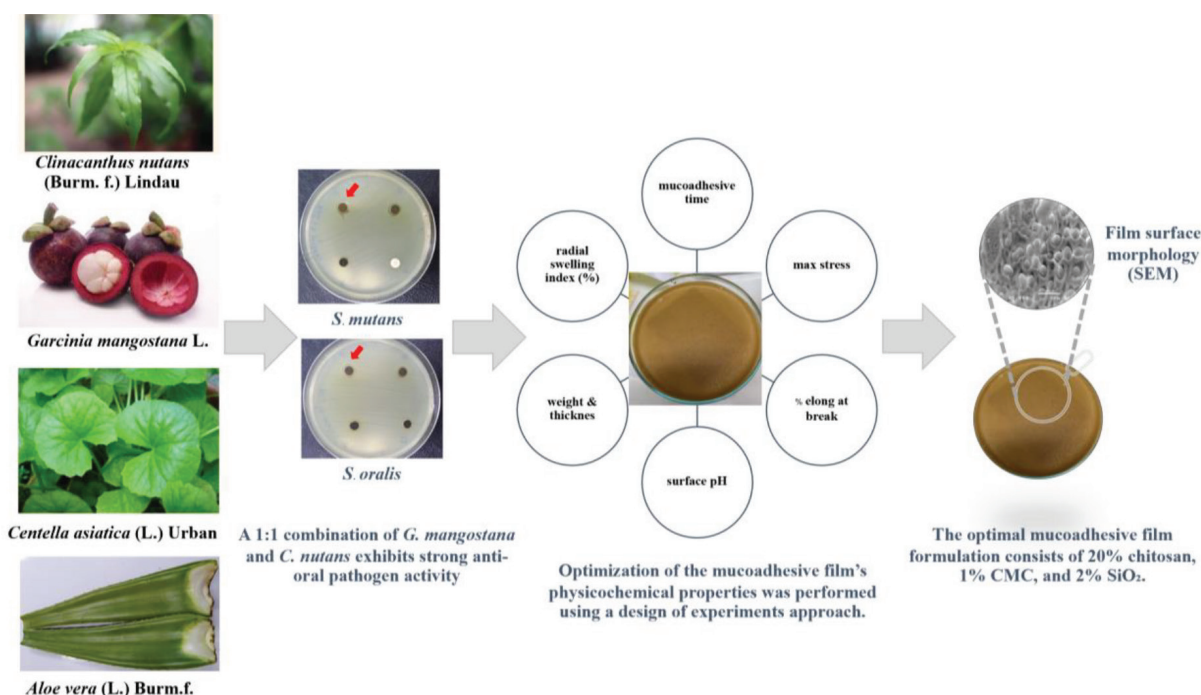
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Abstract

Oral infections caused by *Streptococcus mutans* and *Streptococcus oralis* remain a persistent challenge due to rising antimicrobial resistance and the limitations of synthetic agents. This study explores the antibacterial potential of medicinal herbal extracts and develops a mucoadhesive delivery system for localized oral therapy. A design of experiments (DoE) approach was employed to evaluate the anti-oral pathogen activity of 15 experimental herbal combinations derived from *Garcinia mangostana* L., *Clinacanthus nutans* (Burm. f.) Lindau, *Centella asiatica* (L.) Urban, and *Aloe vera* (L.) Burm. f. The optimal extract combination – a 1:1 ratio of *G. mangostana* pericarp and *C. nutans* leaf extracts – was further incorporated into a mucoadhesive film. A full factorial design was used to optimize the concentrations of three factors: X1, silicon dioxide (SiO₂); X2, chitosan; and X3, carboxymethylcellulose (CMC), to achieve favorable chemical properties and mucosal compatibility. Phytochemical profiling identified α-mangostin, caffeic acid, and ferulic acid as key bioactive compounds. The optimized mucoadhesive film demonstrated favorable chemical properties, sustained mucosal adhesion (≥6 h), and high cytocompatibility (>90% cell viability). Ex vivo buccal mucosa retention studies confirmed transmucosal delivery and retention of active phytochemicals. This study presents a strategy that integrates phytochemical synergy with pharmaceutical technology to address oral infections. The novel mucoadhesive film, incorporating *G. mangostana*–*C. nutans* extracts, offers a promising plant-based platform for localized oral antimicrobial therapy and demonstrates the translational potential of natural actives in advanced drug delivery systems.

Graphical abstract:



Keywords

design of experiments, antibiofilm, *Streptococcus* spp., mangosteen, *Garcinia mangostana*, *Clinacanthus nutans*

Introduction

Oral health problems, particularly dental caries and periodontal diseases, are driven by microbial pathogens such as *Streptococcus mutans* and *Streptococcus oralis*, which play critical roles in oral biofilm formation, acid production, and tissue destruction (Flemming and Wuertz 2019). Conventional treatments, including antibiotics, pose challenges due to antimicrobial resistance, adverse side effects, and potential disruption of the oral microbiome (Ventola 2015). These challenges underscore the urgent need for safe, effective, and natural alternatives to manage oral infections. Herbal extracts, with their rich bioactive profiles, have emerged as promising candidates for combating oral pathogens and reducing biofilm formation (Newman and Cragg 2020).

Several medicinal plants have shown significant potential in this context. Among these, *Garcinia mangostana* L. was selected as an extraction material due to its high content of xanthenes, particularly α -mangostin, which has been well documented for inhibiting biofilm formation and its potent antimicrobial, anti-inflammatory, and antioxidant activities (Pedraza-Chaverri et al. 2008). Xanthenes are primarily concentrated in the pericarp rather than in the pulp or seed, making the pericarp the most

suitable part for obtaining a potent antimicrobial extract (Suksamrarn et al. 2003; FDA Thai: Food and Drug Administration 2013).

Clinacanthus nutans (Burm. f.) Lindau and *Centella asiatica* (L.) Urban were selected for their leaves, which are rich sources of bioactive polyphenols, flavonoids, and triterpenoids, contributing to their well-known antimicrobial, wound-healing, and anti-inflammatory properties. *C. nutans* has gained attention for its broad therapeutic potential in oral health, particularly in managing periodontal and inflammatory conditions. It is rich in polyphenols such as caffeic acid and ferulic acid, which contribute to its antibacterial, antibiofilm, and anti-inflammatory properties (Huang et al. 2016; Kamarudin et al. 2017; Tayeb et al. 2024). Pechroj et al. (2024) demonstrated that nanoemulsified *C. nutans* extract exhibits multifunctional activity, significantly reducing oral pathogens and modulating inflammatory responses relevant to periodontitis. *C. asiatica* contains saponins such as asiaticoside and madecassoside, which possess several biological activities, making it particularly valuable for oral health applications (Singh et al. 2010; Orhan 2012).

Aloe vera (A. vera), applied as a topical gel or mouthwash, has shown significant improvements in various oral mucosal conditions. Patients suffering from aphthous ul-

cers and cancer therapy-induced mucositis have reported reduced pain, decreased inflammation, and faster healing. Moreover, *A. vera* effectively reduces the severity and duration of oral mucositis, improving patient comfort and recovery. Acemannan, a constituent of *A. vera*, exhibits anti-inflammatory, antioxidant, and tissue-repairing properties (Nair et al. 2016; Cássia-Santos et al. 2025). *A. vera* gel also shows strong antibacterial activity against *S. mutans*, a key cariogenic pathogen, demonstrating notable efficacy with a minimum inhibitory concentration (MIC) of 12.5 µg/mL, highlighting its potential as a natural agent for caries prevention (Fani and Kohanteb 2012).

Previous studies have reported the significant antibacterial efficacy of *G. mangostana* and *C. nutans* against oral pathogens, highlighting their potential application in natural oral care therapeutics (Phuong et al. 2017; Suriyaprom et al. 2022). The antimicrobial effects of *G. mangostana* are primarily attributed to α-mangostin, a xanthone derivative known to disrupt bacterial membranes, while *C. nutans* leaves are rich in flavonoids and phenolic compounds that exhibit both antibacterial and anti-inflammatory activities (Zulkipli et al. 2017). Because of their complementary phytochemical profiles, combining these plants may yield synergistic antibacterial effects, enhancing efficacy while reducing dosage requirements. These findings provide the rationale for selecting these extracts for combination studies in this work.

To achieve effective localized delivery in the oral cavity, mucoadhesive films offer a promising platform. They enable prolonged contact time with the buccal mucosa, localized drug release, and improved therapeutic outcomes. The buccal mucosa is recognized as a viable route for delivering both hydrophilic and poorly water-soluble compounds, allowing for localized retention (Yong et al. 2005; Mane et al. 2014).

The development of an optimal mucoadhesive film requires a carefully selected combination of polymers and excipients to ensure both mucoadhesive strength and mechanical stability. Employing a design of experiments (DoE) approach offers a systematic and efficient strategy to optimize such multicomponent formulations, enabling the identification of synergistic interactions among formulation variables while minimizing experimental runs (Montgomery 1984). This method is particularly advantageous for achieving a balanced film with desirable mechanical performance and mucoadhesive properties. In this study, chitosan was selected as the primary adhesive matrix due to its strong mucoadhesive properties and mechanical resilience (Morales and McConville 2011; Ways et al. 2018; Barik et al. 2024). Carboxymethylcellulose (CMC) and silicon dioxide (SiO₂) were incorporated to enhance film flexibility, radial swelling index behavior, and structural integrity (Oliveira et al. 2010; Li et al. 2012).

In this study, we aimed to systematically develop and optimize a mucoadhesive film incorporating active extracts from *G. mangostana*, *C. nutans*, *C. asiatica*, and *A. vera* using a design of experiments (DoE) approach. The formulation was optimized to strike a balance between

mucoadhesive properties and mechanical performance. Additionally, ex vivo porcine buccal mucosa retention studies and cytotoxicity assays were conducted to evaluate the bioavailability and safety of the optimized film. This integrative approach bridges traditional herbal knowledge with modern pharmaceutical technology, providing a pathway for the development of plant-based therapeutics for oral care.

Materials and methods

Medicinal herbal extraction

G. mangostana pericarp and *C. nutans* leaf extraction: Powdered herbal materials were obtained from Vejpongosot Holding Co., Ltd., Thailand, and Don Bong Herbal Group, Prachinburi Province, Thailand. Each plant material was macerated in 95% ethanol (Labscan, Thailand) at a ratio of 1:10 (w/v) for seven days, with gentle agitation twice daily. The mixture was then filtered using a vacuum pump with Whatman No. 1 filter paper, and the ethanol was evaporated using a rotary evaporator (Heidolph Basis Hei-VAP ML Rotary Evaporator, Germany).

C. asiatica leaves extraction: Leaves obtained from Vejpongosot Holding Co., Ltd., Thailand, were subjected to Soxhlet extraction with 95% ethanol (Labscan, Thailand) for 3 days. The solvent was removed using a rotary evaporator (Heidolph Basis Hei-VAP ML Rotary Evaporator, Germany).

A. vera leaves extraction: *A. vera* leaves were collected from Prachuap Khiri Khan Province, Thailand. The inner gel was manually separated from the leaf, washed, homogenized, and freeze-dried using a freeze dryer (Labconco, Missouri, USA).

The extracted compounds were subjected to experimental design using Minitab (version 18, Minitab Inc., USA) to determine the optimal combination for antimicrobial efficacy against *S. mutans* and *S. oralis* (Arminian and Ozgur 2020). The experimental procedure is illustrated in Fig. 1.

Experimental design and optimization of the inhibitory effects on oral pathogens (*S. mutans*, *S. oralis*)

The individual and combined herbal extracts (*G. mangostana*, *C. nutans*, *C. asiatica*, *A. vera*) were optimized using a design of experiments (DoE) approach facilitated by Minitab (version 18, Minitab Inc., USA). A factorial design was selected to systematically evaluate various combinations and interactions of these extracts to determine their optimal antimicrobial efficacy (Chamutpong et al. 2021). The primary factors were the four herbal extracts in different combinations, with each extract either present or absent, coded as 1 or 0, respectively. This resulted in 15 runs, encompassing all possible extract combinations for evaluation, as shown in Table 2. The antimicrobial efficacy of each

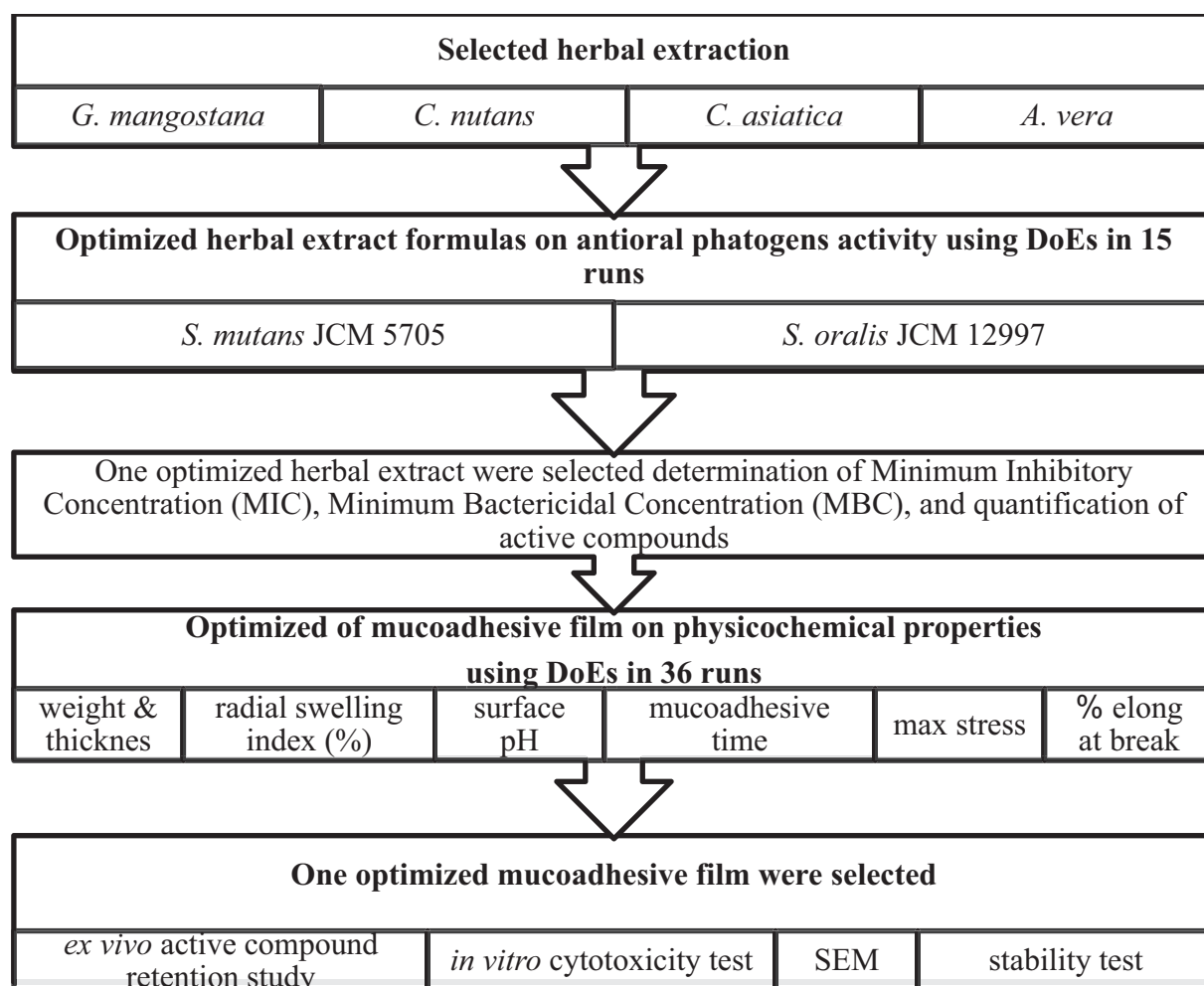


Figure 1. Flowchart of the experimental procedure.

extract combination was evaluated by testing against oral pathogens *S. mutans* and *S. oralis* (Feng et al. 2024). This study assessed the antibacterial activity of herbal extracts against *S. mutans* JCM 5705 and *S. oralis* JCM 12997. Bacteria were grown on brain heart infusion agar (BHIA), adjusted to 0.5 McFarland ($\sim 1 \times 10^8$ CFU/mL), and spread onto BHIA plates. Extracts (10 μ L) were applied to 6 mm sterile discs and placed on the plates, incubated anaerobically at 37 °C for 24 h. DMSO and tetracycline were used as negative and positive controls. Inhibition zones were measured in millimeters. All experiments were performed in duplicate.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The optimal extract combination was selected for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination. Bacterial suspensions (0.1 mL, $\sim 1 \times 10^6$ CFU/mL) were added to 10 mL brain heart infusion broth (BHIB). Extracts (10 mg/mL) were prepared in DMSO, diluted in BHIB, and tested in a 96-well plate using a two-fold serial dilution. DMSO and tetracycline served as negative and positive controls, respectively. A volume of 50 μ L of standardized bacterial suspension was added to 50 μ L of the prepared optimal

extract formula or tetracycline in a 96-well plate. After adding bacterial inoculum, plates were incubated anaerobically at 37 °C for 24 h. Bacterial growth was initially assessed by visual inspection, followed by the addition of 10% Alamar Blue. The MIC was defined as the lowest concentration at which no visible growth was observed. To determine the MBC, 10 μ L from each well showing no visible growth was streaked onto BHIA plates and incubated anaerobically at 37 °C for 24 h. The MBC was defined as the lowest concentration that prevented colony growth on agar plates. These methods were systematically applied to the effective concentrations required to inhibit specific oral pathogens (Thongyim et al. 2024).

Optimization of mucoadhesive films

Mucoadhesive films were formulated using the casting method. A full factorial design was applied in duplicate, varying the formulation for 36 runs (Minitab, version 18). The full factorial design included three factors:

1. Silicon dioxide (SiO_2) (1, 1.5, 2 % w/w)
2. 3% of Chitosan (10, 15, 20% w/w)
3. Carboxymethyl cellulose (CMC) (1, 1.5% w/w).

All formulations are illustrated in Table 1.

Table 1. Inhibitory effects of herbal extracts on *S. mutans* JCM 5705 and *S. oralis* JCM 12997 using the disc diffusion method (duplicate).

run	<i>G. mangostana</i>	<i>C. nutans</i>	<i>C. asiatica</i>	<i>A. vera</i>	Inhibition zone (mm)	
					<i>S. mutans</i> JCM 5705	<i>S. oralis</i> JCM 12997
1	1	0	0	0	6.69 ± 0.03	6.51 ± 0.17
2	0	0	1	0	–	–
3	1	0	1	0	6.72 ± 0.30	6.78 ± 0.29
4	0	0	0	1	–	–
5	1	0	0	1	6.53 ± 0.11	6.51 ± 0.49
6	0	0	1	1	–	–
7	1	0	1	1	6.76 ± 0.01	6.67 ± 0.01
8	0	1	0	0	–	–
9	1	1	0	0	9.37 ± 0.86	9.36 ± 0.43
10	0	1	1	0	–	–
11	1	1	1	0	7.22 ± 0.11	7.07 ± 0.41
12	0	1	0	1	–	–
13	1	1	0	1	8.29 ± 0.51	8.86 ± 0.49
14	0	1	1	1	–	–
15	1	1	1	1	8.06 ± 0.89	7.62 ± 0.78
16 Tetracycline (- control)	0	0	0	0	8.26 ± 0.65	9.15 ± 1.10

Preparation of mucoadhesive films

SiO₂ was dissolved in 60–80 g PEG600 in a water bath. The 16-fold MIC extract from the previous experiment was mixed with 5 g of glycerin and 1.5 g of sorbitol (Phase I) (Limboo and Singh 2024). Carboxymethylcellulose and chitosan solution were then added using a homogenizer at 6000 rpm for 10 min (Phase II). Phases I and II were mixed until homogeneous and dried in a hot-air oven at 60 °C for 12 h.

Chemical properties of mucoadhesive film:

- **Thickness and weight**
The weight of the film was measured using a digital analytical balance (4 decimals, Mettler Toledo, Switzerland). Thickness was measured using a vernier micrometer (Digital Offset Caliper Vernier, Mitutoyo, Japan).
- **Radial swelling index**
A mucoadhesive film, 2 cm in diameter, was allowed to swell on the surface of an agar plate and then placed in an incubator maintained at 37 °C. The diameter of the swollen film was measured at predetermined time intervals for 90 min. The radial swelling index was calculated using the following equation:

$$\text{Radial swelling index (\%)} = [(D_t - D_0) / D_0] \times 100]$$

where D_t is the diameter of the swollen film after time t , and D_0 is the original film diameter at time zero (Nafee et al. 2003).

- **Mucoadhesive film surface pH**
The surface pH of the mucoadhesive film was determined using a pH meter (pH meter S400, Mettler Toledo, Switzerland). The film was placed on a Petri dish containing 5 mL of deionized water and allowed to swell for 1 h at room temperature. The pH

was measured by bringing a combined glass electrode into contact with the surface of the swollen film and allowing it to equilibrate for 1 min. The average of three readings was recorded (Chaiprateep et al. 2018).

- **Puncture resistance test**
The puncture resistance test measured maximum stress and elongation at break of the film using a texture analyzer (TA.XT, Stable Micro System Co., Ltd., England). The experimental conditions included a load cell of 30 kg, spherical probe P/5S, contact force of 5 g, and a 0.30 mm/sec test speed. Testing was conducted on films of size 2.25 cm². Maximum stress, Young's modulus (MPa), and elongation at break (%) were recorded. All experiments were performed in triplicate (Landová et al. 2014).
- **Ex vivo mucoadhesive retention time**
A modified USP disintegration apparatus lined with porcine buccal mucosa was used as the experimental model. The mucosa was rinsed with deionized water and then with simulated saliva (pH 6.2) at room temperature. The porcine buccal mucosa was affixed to a glass slide, and the wet film, previously moistened with one drop of simulated saliva, was applied to the mucosal surface using gentle fingertip pressure for 10 seconds. The glass slide was positioned vertically in the apparatus and allowed to move up and down, with its lowest point extending beyond the surface at the highest position. The beaker was filled with 800 mL of simulated saliva. The time required for the film to detach from the mucosa was recorded (Vasanthi et al. 2011).
- **Scanning electron microscope (SEM)**
The mucoadhesive film was sectioned into 1 cm² samples and mounted onto aluminum stubs. To enhance conductivity, samples were sputter-coated with gold for 10 seconds. Cross-sectional morphology was examined using a scanning electron microscope (model JEOL, JSM 840, Tokyo, Japan) (Vry et al. 2020).

Ex vivo active compound buccal mucosa retention study

This study evaluated the ex vivo retention of active compounds through porcine buccal mucosa using a Franz diffusion cell (Garcia-Tarazona et al. 2023). Fresh buccal porcine mucosa was obtained from a slaughterhouse. The tissue was placed between the donor and receptor compartments and clamped together. The receptor compartment was filled with isotonic phosphate buffer (pH 7.4). The diffusion cell was maintained at 37 ± 2 °C, and the receptor compartment was continuously stirred throughout the experiment. After 6 h, the film samples were gently removed from the buccal mucosa, which was then collected. The active compounds retained in the buccal mucosa were determined by homogenizing the tissue with methanol to extract the penetrated compounds. The sample was filtered, and the concentration of phytochemicals was analyzed using HPLC.

Quantification of active compounds of herbal extract using a high-performance liquid chromatography (HPLC) method

This HPLC method quantified key active compounds in the optimized Thai herbal extract. Analysis was performed on a ZORBAX Eclipse Plus-C18 column (4.6×100 mm, $5 \mu\text{m}$) at 30 °C with a 0.8 mL/min flow rate, $20 \mu\text{L}$ injection volume, and a 30-minute run time. Detection of caffeic acid, ferulic acid, and α -mangostin was carried out at 320 nm. The mobile phase consisted of a gradient of potassium dihydrogen phosphate buffer (pH 2.5) and acetonitrile. Calibration curves were prepared individually for caffeic acid (1.25 – $20 \mu\text{g/mL}$), ferulic acid (10 – $70 \mu\text{g/mL}$), and α -mangostin (25 – $125 \mu\text{g/mL}$), each demonstrating high linearity ($R^2 > 0.99$), thereby ensuring accurate quantification of target compounds. Results are expressed as $\mu\text{g/mL}$.

In vitro cytotoxicity assay

The cytotoxicity of the mucoadhesive film was evaluated using a fibroblast cell line, based on the protocol adapted from Ninan et al. (2016) with modifications. Mucoadhesive film precursor solutions ($100 \mu\text{L}$) were dispensed into 96-well plates and allowed to form films. After cross-linking, the films were sterilized with 70% ethanol and rinsed with sterile $1\times$ phosphate-buffered saline (PBS). Each film sample was incubated in 1 mL of Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and $100 \mu\text{g/mL}$ streptomycin at 37 °C in a humidified 5% CO_2 atmosphere for 24 h to obtain the mucoadhesive film extract. NIH 3T3 fibroblast cells (American Type Culture Collection, Rockville, MD, USA; ATCC® CRL-1658™) (1×10^4 cells/well) were seeded into 96-well plates and cultured under identical conditions for 24 h. The culture medium was then replaced with $100 \mu\text{L}$ of the mucoadhesive film extract, and the cells

were incubated for an additional 24 h. Cell viability was assessed using the MTT assay. A 0.5 mg/mL MTT solution was added to each well and incubated in the dark for 4 h. The resulting formazan crystals were dissolved in a solubilization buffer, and the plate was incubated for another 2 h. Absorbance was measured at 570 nm using a microplate reader. Cell viability was expressed as a percentage relative to the untreated control (Ninan et al. 2016).

Accelerated stability test

The selected mucoadhesive films were studied for accelerated stability by wrapping them in aluminum foil. The films were kept in an incubator maintained at 45 ± 0.5 °C for 24 h, then switched to 8 ± 0.5 °C for 24 h for five cycles. The mucoadhesive films were examined for changes in color, shape, and odor (Ways et al. 2018).

Data analysis and optimization

Statistical analysis was conducted to confirm the significance of the results. Data from each combination were analyzed in Minitab to assess the main effects, interaction effects, and the optimal combination of extracts for maximum antimicrobial activity. ANOVA was performed with a significance level of 0.05 to identify statistically significant factors and interactions (Minitab, version 18, USA) (Montgomery 1984).

Results and discussion

Optimization of the inhibitory effects on oral pathogens (*S. mutans*, *S. oralis*)

The preparation of *C. nutans*, *C. asiatica*, *G. mangostana*, and *A. vera* extracts resulted in yields of approximately 5% for each extract, ensuring that all extracts were standardized to the same concentration for further study. The optimal combination of herbal extracts was found to target *S. mutans* and *S. oralis*. A combination of *G. mangostana* and *C. nutans* exhibited potent antibacterial activity. In the disc diffusion assay, this optimized combination produced inhibition zones of 8.720 ± 0.651 mm for *S. mutans* and 9.145 ± 0.091 mm for *S. oralis*. Although these values were slightly lower than those achieved with tetracycline, the positive control (9.365 ± 0.856 mm and 9.355 ± 0.431 mm, respectively), the herbal combination still yielded statistically significant inhibition ($p < 0.05$), as detailed in Table 1.

Further optimization using Minitab's desirability function analysis (Fig. 2) confirmed this binary combination as the most effective among the combinations tested. The optimal combination of *G. mangostana* and *C. nutans* achieved a high composite desirability score of 0.9496 – approaching the ideal value of 1.0. Specifically, the desirability values for inhibition against *S. mutans* and *S. oralis* were 0.93932 ($y = 9.3650$ mm) and 0.93785 ($y = 9.3550$ mm), respectively. This indicates that the opti-

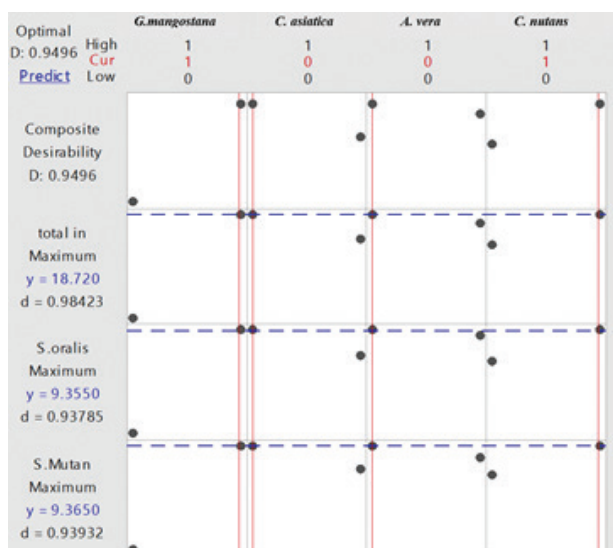


Figure 2. The optimal combination of herbal extracts for anti-oral pathogen efficiency.

mized formula successfully meets the desired antibacterial performance criteria for both target organisms.

These results suggest a strong synergistic interaction between the phytochemical constituents of *G. mangostana* and *C. nutans* compared with single-extract combinations. Such synergy is essential in developing multicomponent herbal interventions, particularly for controlling oral pathogens, where the complexity of biofilms and microbial interactions requires multi-targeted mechanisms of action.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) from optimized herbal extract combinations and quantification of active compounds

The optimized 1:1 combination of *G. mangostana* pericarp and *C. nutans* leaf extracts demonstrated potent anti-oral pathogen effects against *S. mutans* and *S. oralis*, with an MIC of 15.62 µg/mL and an MBC of 62.5 µg/mL. Specifically, α-mangostin – an abundant xanthone in *G. mangostana* – along with caffeic acid and ferulic acid – two key polyphenols in *C. nutans* – have been shown to exert antimicrobial effects at relatively low concentrations. The current findings highlight the potential of combining these phytochemicals to enhance antibacterial efficacy through synergistic interactions (Kaomongkolgit et al. 2009; Zulkipli et al. 2017). Optimization strategies, particularly those employing a design of experiments (DoE) approach, as demonstrated by Saelee et al. (2023), have proven effective in identifying synergistic herbal combinations that maximize bioactivity while minimizing required dosages. In this context, our study highlights the value of targeted phytochemical pairing to enhance antimicrobial performance, supporting the development of natural, plant-based alternatives to conventional antibiotics in oral healthcare (Saelee et al. 2023).

Key compounds were quantified to elucidate the contribution of individual bioactive constituents within the optimized combination. In the most effective herbal extracts, which utilized a 1:1 ratio of *G. mangostana* and *C. nutans*, the concentrations of caffeic acid (273.76 ± 0.15 µg/mL), ferulic acid (23.20 ± 0.30 µg/mL), and α-mangostin (9.92 ± 0.23 ng/mL) were determined. These concentrations are consistent with levels previously associated with antimicrobial activity, further validating their role in the observed inhibition of oral pathogens. Caffeic and ferulic acids have been shown to disrupt bacterial cell membranes, inhibit nucleic acid synthesis, and prevent biofilm formation – key mechanisms underlying their antimicrobial actions (Sivaranjani et al. 2018). α-Mangostin, in particular, is known for its ability to permeabilize bacterial membranes and has demonstrated efficacy against various *Streptococcus* species due to its potent lipophilic structure and antioxidant properties (Pedraza-Chaverri et al. 2008).

These findings support the hypothesis that combining polyphenolic acids from *C. nutans* with xanthones from *G. mangostana* produces a synergistic antibacterial effect greater than that of individual components. Such synergy is crucial for developing multitargeted botanical therapies capable of overcoming microbial resistance and disrupting complex oral biofilms. Therefore, this study reinforces the potential of rationally optimized, phytochemical-rich herbal combinations as viable and effective alternatives to synthetic antimicrobial agents for managing oral infections (Saelee et al. 2023).

Optimization of the mucoadhesive film formulation

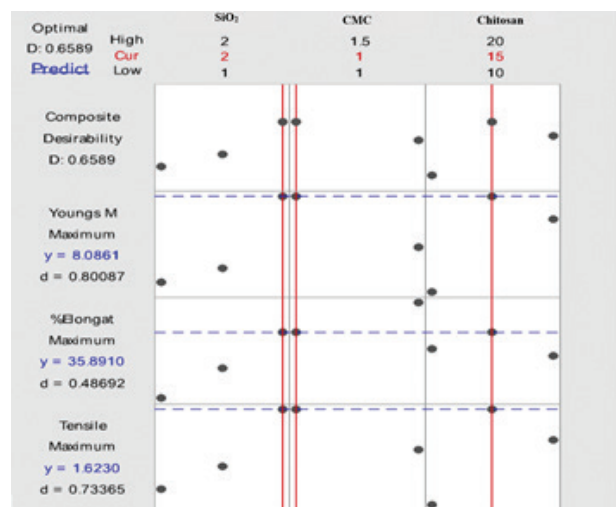
Thirty-six experimental runs were analyzed using a design of experiments (DoE) approach (Table 2). The resulting films exhibited thicknesses ranging from 0.7 to 1.7 mm. Surface pH values across all formulations were between 6.5 and 7.5, close to salivary pH in the oral cavity (6.5–7.5). This indicates that the prepared mucoadhesive film of *C. nutans* and *G. mangostana* can be applied without causing mucosal irritation. Each formulation demonstrated a mucoadhesive retention time exceeding 6 h, affirming their potential for prolonged contact with the mucosal surface. The optimal formulation was identified through a design of experiments analysis, as shown in Table 2 and Fig. 3. Several parameters were evaluated, including tensile strength, mucoadhesion time, radial swelling index (%), and surface pH. The formulation containing 2% SiO₂, 1% CMC, and 15% chitosan offered the best overall balance, showing strong yet flexible films, sustained mucoadhesion for more than 6 h, and a near-oral cavity pH.

The incorporation of SiO₂ served as structural reinforcement within the polymeric matrix. SiO₂ enhanced mechanical properties by increasing both tensile strength and Young's modulus, owing to strong interfacial interactions between the nanoparticles and polymer chains (Oliveira et al. 2010; Giri et al. 2012). This reinforcement is essential for maintaining the integrity of the film under mechanical stress during application and continuous contact within the dynamic oral

Table 2. Chemical properties of mucoadhesive film.

run	X1:SiO2 (%w/w)	X2:Chitosan (%w/w)	X3:CMC (%w/w)	Max stress	Young's modulus (Mpa)	Elong at break (%)
F1	1	10	1	0.31 ± 0.02	1.36 ± 0.10	43.17 ± 1.96
F2	1	15	1	0.18 ± 0.02	0.80 ± 0.06	37.10 ± 0.17
F3	1	20	1	0.11 ± 0.01	0.62 ± 0.08	24.85 ± 0.25
F4	1	10	1.5	0.18 ± 0.01	0.54 ± 0.11	59.61 ± 0.31
F5	1	15	1.5	0.33 ± 0.06	1.81 ± 0.13	35.42 ± 0.57
F6	1	20	1.5	0.10 ± 0.01	25.60 ± 0.71	211.88 ± 8.07
F7	1.5	10	1	0.15 ± 0.02	0.97 ± 0.10	25.76 ± 5.37
F8	1.5	15	1	0.03 ± 0.00	0.66 ± 0.40	11.95 ± 2.21
F9	1.5	20	1	0.02 ± 0.01	0.85 ± 0.22	20.89 ± 5.03
F10	1.5	10	1.5	0.08 ± 0.00	0.34 ± 0.01	31.06 ± 1.66
F11	1.5	15	1.5	0.08 ± 0.01	0.99 ± 0.117	37.70 ± 1.99
F12	1.5	20	1.5	0.08 ± 0.01	0.54 ± 0.03	19.29 ± 0.90
F13	2	10	1	0.16 ± 0.02	1.01 ± 0.09	31.30 ± 5.76
F14	2	15	1	0.14 ± 0.01	0.43 ± 0.06	48.48 ± 3.09
F15	2	20	1	0.13 ± 0.01	0.41 ± 0.01	43.05 ± 5.85
F16	2	10	1.5	0.10 ± 0.01	0.57 ± 0.29	32.77 ± 0.98
F17	2	15	1.5	0.16 ± 0.03	1.21 ± 0.29	27.18 ± 6.21
F18	2	20	1.5	0.11 ± 0.00	0.50 ± 0.19	25.29 ± 4.87
F19	1	10	1	0.22 ± 0.01	1.23 ± 0.21	32.17 ± 2.55
F20	1	15	1	0.16 ± 0.01	1.04 ± 0.03	28.50 ± 2.91
F21	1	20	1	0.11 ± 0.01	0.64 ± 0.14	21.70 ± 6.40
F22	1	10	1.5	0.26 ± 0.04	0.63 ± 0.05	65.90 ± 11.70
F23	1	15	1.5	0.32 ± 0.03	1.60 ± 0.10	36.72 ± 0.38
F24	1	20	1.5	0.13 ± 0.02	0.38 ± 0.11	172.00 ± 37.59
F25	1.5	10	1	0.15 ± 0.01	0.94 ± 0.05	28.43 ± 1.69
F26	1.5	15	1	0.02 ± 0.00	0.29 ± 0.07	13.79 ± 1.27
F27	1.5	20	1	0.01 ± 0.00	0.18 ± 0.02	20.33 ± 4.53
F28	1.5	10	1.5	0.09 ± 0.01	0.56 ± 0.05	33.60 ± 8.58
F29	1.5	15	1.5	0.20 ± 0.00	0.99 ± 0.17	37.20 ± 0.86
F30	1.5	20	1.5	0.11 ± 0.01	0.63 ± 0.07	22.32 ± 0.35
F31	2	10	1	0.16 ± 0.00	1.00 ± 0.14	25.94 ± 2.15
F32	2	15	1	0.08 ± 0.01	0.33 ± 0.01	41.19 ± 2.89
F33	2	20	1	0.13 ± 0.01	0.36 ± 0.01	53.68 ± 4.41
F34	2	10	1.5	0.10 ± 0.00	0.60 ± 0.25	28.22 ± 0.70
F35	2	15	1.5	0.14 ± 0.01	1.19 ± 0.32	22.95 ± 4.41
F36	2	20	1.5	0.10 ± 0.01	0.39 ± 0.03	37.45 ± 4.79

cavity (Pooja et al. 2024). However, excessive SiO₂ loading can lead to agglomeration, resulting in poor film uniformity and reduced mechanical strength. Thus, its concentration must be carefully optimized (Oliveira et al. 2010). CMC, a hydrophilic, anionic cellulose derivative, plays a dual role in the formulation, acting as both a plasticizer and a flexibility enhancer. Its ability to absorb water promotes swelling and facilitates close contact with the mucosal surface through hydrogen bonding, which is a key mechanism for effective mucoadhesion (Schattling et al. 2017). Within the chitosan matrix, CMC disrupts the rigid interactions among chitosan chains, thereby increasing elongation at break and improving film flexibility (Li et al. 2012; Valizadeh et al. 2019). However, excessive CMC content can compromise tensile strength, emphasizing the importance of fine-tuning its concentration to achieve the desired balance between elasticity and mechanical stability (Alves et al. 2020).

**Figure 3.** The optimal plot of mucoadhesive film formulation.

Chitosan, a biocompatible, cationic polysaccharide, provides the essential adhesive base for the mucoadhesive film. Its positive charge enables strong electrostatic interactions with negatively charged mucin, enhancing mucoadhesion (Morales and McConville 2011; Ways et al. 2018). Additionally, chitosan contributes to the film's overall mechanical strength. However, at higher concentrations, it can lead to increased brittleness, as reflected in decreased elongation at break values (Barik et al. 2024). The optimized formulation benefits from the synergistic integration of these three components. This combination yields a mucoadhesive film with excellent mucoadhesive properties, structural integrity, and compatibility for sustained oral delivery of herbal actives.

The present findings are consistent with previous reports demonstrating the potential of herbal-based mucoadhesive films for oral applications. For example, Mythri et al. (2011) developed a chitosan-based mucoadhesive film incorporating herbal extracts and observed prolonged mucoadhesive time and effective localized delivery, though their study utilized single-plant extracts. Similarly, Saelee et al. (2023) employed a design of experiments (DoE) approach to optimize herbal combinations in oral care formulations, underscoring the value of systematic optimization for enhancing synergistic antibacterial effects. Compared to these studies, our formulation uniquely combines four complementary herbal extracts – *G. mangostana*, *C. nutans*, *C. asiatica*, and *A. vera* – which provide a broader spectrum of bioactivities, including antibacterial, anti-inflammatory, and wound-healing effects. This multi-extract approach represents a novel strategy that leverages phytochemical synergy to enhance efficacy while maintaining biocompatibility. In addition, our film achieved a mucoadhesive retention time exceeding 6 h, comparable to or exceeding the performance reported in previous works, with favorable mechanical properties and ex vivo mucosal retention of active compounds (Barik et al. 2024).

Ex vivo active compound buccal mucosa retention study and cytotoxicity test

Following a 4-h application in the Franz diffusion cell system, analysis of residual porcine buccal mucosa tissue revealed significant retention of the active compounds: caffeic acid ($235.28 \pm 0.15 \mu\text{g/mL}$), ferulic acid ($19.4 \pm 0.3 \text{ ng/mL}$), and α -mangostin ($8.54 \pm 23.46 \mu\text{g/mL}$). This finding is consistent with the intended objective of developing a localized, topical treatment that does not require systemic absorption. Maintaining the active compounds within the buccal mucosa enables prolonged interaction at the target site, thereby enhancing their potential antimicrobial efficacy while minimizing the risk of systemic exposure.

The presence of α -mangostin – a lipophilic xanthone – in the receptor phase indicates its ability to permeate the buccal mucosa, aligning with previous reports on enhanced mucosal lipophilic permeation (Mane et al. 2014). In contrast, caffeic acid and ferulic acid, both hydrophilic

phenolic acids, were primarily retained within the mucosal tissue, supporting the role of the buccal mucosa in localized delivery and tissue retention of hydrophilic phytochemicals (Yong et al. 2005).

The dual disposition – systemic absorption of α -mangostin alongside localized retention of caffeic and ferulic acids – underscores the multifunctional capability of the optimized mucoadhesive film. It not only facilitates transmucosal drug delivery but also offers potential for localized therapeutic effects at the site of oral infection or inflammation. These outcomes highlight the critical role of polymeric matrix design in modulating both the release kinetics and mucosal interaction of bioactive compounds.

Moreover, cytotoxicity testing of the optimized formulation showed >90% cell viability at all time points (24, 48, and 72 h), confirming its excellent biocompatibility. This is consistent with prior evidence supporting the safety of SiO_2 , chitosan, and CMC for mucosal drug delivery applications (Li et al. 2012). These materials are known for their non-toxic profiles, soft tissue compatibility, and low irritation potential when administered via the buccal mucosa route.

Stability testing under accelerated conditions revealed no significant changes in the films' color, shape, or odor, indicating good physical stability. Furthermore, scanning electron microscopy (SEM) images (Fig. 4) revealed a uniform surface morphology and a well-integrated matrix in both top and cross-sectional views. These structural characteristics enhance the film's physical robustness and ensure a consistent distribution of actives. Collectively, these findings strongly support that the selected polymeric composition – 20% chitosan, 1% CMC, 2% SiO_2 – effectively balances physical properties, mucoadhesion, buccal mucosa retention, and biocompatibility. The optimized mucoadhesive film (Fig. 5) thus represents a promising delivery platform for natural anti-oral pathogen agents, supporting both localized and systemic therapeutic strategies in oral healthcare.

Conclusion

This study highlights the potent antibacterial and antibiofilm efficacy of a 1:1 combination of *G. mangostana* pericarp and *C. nutans* leaf extracts against *S. mutans* and *S. oralis*, two major contributors to oral infections. The optimized combination of herbal extracts produced inhibition zones nearly equivalent to those of tetracycline and achieved an MIC of $15.62 \mu\text{g/mL}$, underscoring its potential as a natural, herbal-derived alternative in oral care. The therapeutic effect is primarily attributed to the synergistic action of its major bioactive compounds – α -mangostin, caffeic acid, and ferulic acid – all of which possess well-documented antimicrobial and anti-inflammatory activities. These compounds inhibited bacterial growth and demonstrated promising retention in buccal mucosa tissue, suggesting both localized and systemic therapeutic potential.

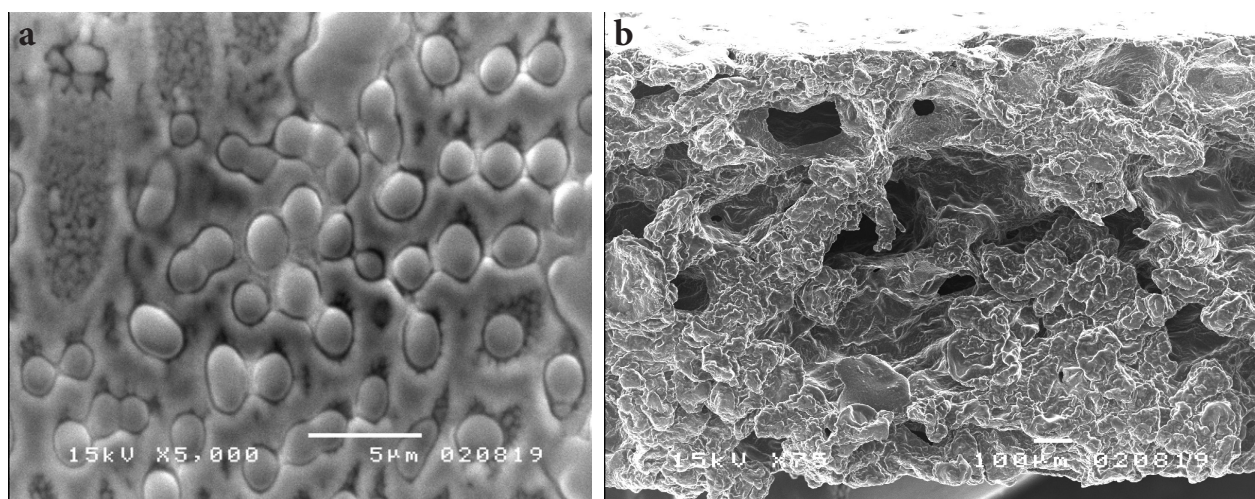


Figure 4. SEM images of the top view (a) and cross-sectional view (b).



Figure 5. The optimized mucoadhesive film.

Employing a design of experiments (DoE) and desirability function analysis enabled the precise formulation and optimization of film composition. The formulation was optimized to maximize anti-oral pathogen activity while maintaining critical parameters such as chemical properties, adhesiveness, biocompatibility, and film stability. Additionally, this study exemplifies the integration of phytochemical knowledge with advanced formulation strategies. The resulting mucoadhesive delivery system offers a novel, effective, evidence-based intervention for managing oral infections within integrative medicine. An acknowledged limitation of this study is the absence of a comprehensive release profile and solubility assessment under simulated saliva conditions. These aspects will be systematically addressed in future investigations to enhance scientific understanding and optimize the clinical performance of the developed film.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Use of AI

No use of AI was reported.

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Author contributions

Conceptualization, methodology, formal analysis, Chaiprateep; investigation, all authors: resources, Chaiprateep; data curation, Chaiprateep; writing – original draft preparation, all authors; writing – review and editing, all authors: visualization, Chaiprateep; All authors have read and agreed to the published version of the manuscript.

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- ## Data availability
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