

Formulation And Evaluation Of Mucoadhesive Oral Film Containing Clove Oil And Green Tea Extract For Dental Caries

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Abstract

Background: Dental caries is a common oral disease mainly associated with cariogenic bacteria such as *Streptococcus mutans* and *Lactobacillus acidophilus*. The present study was carried out to develop a mucoadhesive oral film containing green tea extract and clove oil for local application in dental caries. **Materials and Method:** Green tea was extracted using 70% ethanol and screened for phytoconstituents. Oral films were prepared by solvent casting using HPMC E15 and Carbopol 934P. Design Expert[®] software was used to study the effect of polymer concentrations. The films were evaluated for physical properties, pH, folding endurance, swelling, TPC, TFC, mucoadhesive strength, drug content, antimicrobial activity, drug release and stability. **Result and Discussion:** The optimized film showed good flexibility, acceptable pH and uniform drug content. Mucoadhesive strength was found to be 0.268 ± 0.02 N. The formulation produced inhibition zones of 16 mm against *S. mutans* and 14 mm against *L. acidophilus*. The film showed gradual release of the active constituents and remained stable during three months of storage. Overall, the findings indicate that the prepared green tea extract and clove oil film has suitable properties for localized oral application and may be useful in dental-caries management.

Keywords: Dental caries, mucoadhesive oral film, green tea extract, clove oil, TPC, TFC.

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INTRODUCTION

Dental caries is a common problem of the oral cavity and is mainly associated with bacteria such as *Streptococcus mutans* and *Lactobacillus acidophilus*¹. These bacteria produce acids that contribute to the breakdown of tooth structure. Most oral preparations are used to control bacterial growth and maintain oral hygiene. However, medicines placed in the mouth can be washed away quickly by saliva or swallowed, reducing their contact with the affected area. A formulation that stays in the mouth for a longer time can therefore be useful for local treatment. Mucoadhesive films are suitable for this purpose because they can stick to the mucosal surface and provide local delivery of active ingredients^{2,3}.

Green tea (*Camellia sinensis*)^{4,5} and clove (*Syzygium aromaticum*)⁷ were selected for the present study. Green tea contains phenolic compounds, flavonoids, tannins and other constituents that have reported antimicrobial properties. Clove oil contains eugenol, which is known for its activity against different microorganisms. The phytochemical screening carried out in this study also showed the presence of phenolic compounds and flavonoids in the selected plant materials. These properties made green tea extract and clove oil suitable candidates for a dental-caries formulation⁸.

Mouthwashes, gels and other oral preparations are commonly used for maintaining oral health and controlling microorganisms. However, their contact with the oral mucosa is often short because of saliva, swallowing and normal movements of the mouth. Liquid preparations are also difficult

to keep at one particular site. Because of this, repeated application may be needed to maintain the local effect. These problems show the need for a dosage form that can remain at the required site for a longer period and release its active ingredients gradually⁹.

A mucoadhesive oral film containing green tea extract and clove oil was therefore developed. HPMC E15 and Carbopol 934P were used to form the film and provide adhesion to the mucosa, while propylene glycol improved flexibility and Tween 80 helped in mixing the clove oil. The films were prepared by solvent casting and evaluated for their physical properties, pH, folding endurance, swelling, mucoadhesive strength, drug content, antimicrobial activity, drug release and stability¹⁰.

MATERIALS AND METHODS

a. Collection and authentication of plant

The dried leaves of green tea extract (*Camellia sinensis*) were procured from a local herbal market of Solapur, Maharashtra. The clove oil (*Syzygium aromaticum*) is issued by Department of Pharmacognosy, SVERI College of Pharmacy, Pandharpur. The identification of green tea was carried by the Department of Botany K.B.P. Mahavidhyalaya, Pandharpur.

b. Extraction of plant material

Fresh green tea leaves were washed with distilled water and shade dried for 48–72 hours. The dried leaves were powdered and 100 g was extracted with 70% ethanol using a Soxhlet for 6–8 hours. The extract was filtered with

Whatmann filter paper no.1, concentrated using rotatory evaporator, dried at 40–45°C, and stored in an airtight container at 4°C until further use¹².

c. Preliminary phytochemical screening

The identification of phytochemicals of green tea extract and clove oil was carried for alkaloids, Saponins, tannins, glycosides, anthraquinone, amino acid, phenols, and flavonoids¹³.

d. Total phenolic content determination

Total phenolic content was determined by Folin-Ciocalteu's method. 1 ml of aliquots and standard gallic acid (10-100 µg/ml) was positioned into the test tubes and 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent was mixed and shaken. After 5 minutes, 1.5 ml of 20 % sodium carbonate was added and volume made up to 10 ml with distilled water. Incubated tubes for 2 hours at room temperature. After incubation intense blue color was developed and absorbance was measured at 750 nm using UV – visible spectrophotometer. The extracts were performed in triplicates. The blank was performed using reagent blank with solvent. Gallic acid was used as standard. The calibration curve was plotted using standard gallic acid. The data for total phenolic contents of polyherbal formulation were expressed as mg of gallic acid equivalent weight (GAE)/ 100 g of dry mass^{14,15}.

e. Total flavonoid content determination

Total flavonoid content was measured using the aluminium chloride colorimetric assay. 1ml of aliquots and 1ml standard quercetin solution (100-1000 µg/ml) was positioned into test tubes and 4ml of distilled water and 0.3 ml of 5 % sodium nitrite solution was added into each tubes. After 5 minutes, 0.3 ml of 10 % aluminum chloride was added. At 6th minute, 2 ml of 1 M sodium hydroxide was added. Finally, volume was making up to 10 ml with distilled water and mix well. Orange yellowish color was developed. The absorbance was measured at 510 nm spectrophotometer using UV-visible Jasco V-630 instrument. The blank was performed using distilled water. Quercetin was used as standard. The samples were performed in triplicates. The calibration curve was plotted using standard quercetin. The data of total flavonoids of polyherbal formulation were expressed as mg of quercetin equivalents/ 100 g of dry mass^{14,15}.

f. In vitro antioxidant assay

In vitro antioxidant assay was estimated by ferric reducing antioxidant power (FRAP) method. To the 2.5 ml of extract, 1ml of 0.2 M phosphate buffer pH 6.6 and 1 ml of 1 % potassium ferricyanide was added. The reaction mixture was incubated in water bath at 50 °C for 20 minutes. Afterward, reaction mixture was rapidly cooled and 2.5 ml of 10 % trichloroacetic acid was added to stop the reaction and was centrifuged for 10 minutes. 2.5 ml of aliquots was pipetted out and 2.5 ml of distilled water and 0.5 ml of 0.1 % ferric chloride solution was added. The color changes to green. The mixture was allowed to stand for 10 minutes and absorbance was measured at 593 nm spectrophotometrically using UV- visible Jasco V-630 instrument. The blank was performed using reagent blank and solvent. Ascorbic acid was used as standard. The extracts were performed in triplicate¹⁴.

g. Antimicrobial Activity

Antimicrobial activity was tested by the agar well diffusion method. Sterile agar was mixed with the test microorganism and poured into sterile Petri plates. After the agar solidified, 6 mm wells were made using a sterile cork borer. The test sample was prepared at a concentration of 10 mg/mL. About 100 µL of each sample was added to the respective wells. Standard solution (1 mg/mL) was used as the positive control, while DMSO was used as the negative control. The plates were incubated under suitable conditions. After incubation, the zone of inhibition was measured in millimetres¹⁶.

h. Preparation of cream using DoE

The mucoadhesive oral film was prepared by solvent casting. HPMC E15 and Carbopol 934P were dispersed separately in distilled water and allowed to hydrate. The two solutions were then mixed. Propylene glycol was added to make the film flexible, and Tween 80 was used to mix the clove oil properly. Green tea extract, clove oil, citric acid, and sodium saccharin were added slowly with stirring. The mixture was stirred for 30–45 minutes and sonicated to remove air bubbles. It was poured into a glass mould and dried at 40–45°C for 18–24 hours. The dried film was peeled off, cut into 2 × 2 cm² pieces, wrapped in aluminium foil, and stored¹⁸.

Table no. 01 Code levels of Design Expert software

Independent variables	Coded levels		
X1 (HPMC)	-1	0	+1
X2 (Carbopol)	-1	0	+1

Table no.02 Experimental runs by 2² factorial design in DoE

Formulation	X1 HPMC (%)	X2 Carbopol (%)
1	0.25	0.04

2	0.35	0.04
3	0.25	0.06
4	0.35	0.06
5	0.25	0.05
6	0.35	0.05
7	0.3	0.04
8	0.3	0.06
9	0.3	0.05
10	0.3	0.05
11	0.3	0.05

i. Evaluation of film parameters

a. Physical examination

The buccal films were checked for colour, transparency, smoothness, uniformity, and flexibility. Thickness was measured at five different points using Vernier calliper. The average thickness was then calculated to check whether the films had uniform thickness¹⁹.

b. Surface pH

The surface pH of the buccal films was checked to ensure it was suitable for the buccal cavity and less likely to cause irritation. The films were soaked in 5 mL distilled water for 2 hours. After swelling, the surface pH was measured using a calibrated digital pH meter^{19,20}.

c. Folding Endurance

Folding endurance was checked to assess the strength and flexibility of the buccal films. Each 2×2 cm² film was folded repeatedly at the same spot by hand until it broke or showed visible cracks. The number of folds was recorded. A folding endurance above 200 indicates good mechanical strength for buccal application¹⁹.

d. Mucoadhesive strength

Mucoadhesive strength was measured using a modified physical balance method with freshly excised porcine buccal mucosa. The mucosa was fixed on a glass slide and kept in phosphate buffer (pH 6.8). The film was placed against the mucosa for 2 minutes under light pressure. Weight was gradually added until the film detached. Force required for detachment of film from mucosa was recorded¹⁹.

e. Drug Content Uniformity

Drug content uniformity was checked to ensure each film contained the required amount of drug. Each film was dissolved in 100 mL phosphate buffer (pH 6.8) and sonicated for 15 minutes. The solution was filtered and its absorbance measured at 278 nm for green tea extract and 282 nm for clove oil¹⁹.

f. In-Vitro Release Test

In vitro drug release studies were carried out using a Franz diffusion cell with a receptor compartment volume of 15 mL filled with phosphate buffer (pH 6.8) maintained at $37 \pm 0.5^\circ\text{C}$ and stirred at 100 rpm. A dialysis membrane (MW cutoff 12,000 Da) was used to separate the donor and receptor compartments. The film was placed on the donor side, and aliquots (5 mL) were withdrawn at predetermined intervals (0.5–6 hours) and replaced with fresh buffer. Samples were filtered and analyzed at 278 nm for green tea extract. The cumulative percentage of drug release was plotted against time to determine the release kinetics (zero-order, first-order, Higuchi, and Korsmeyer–Peppas models)²¹.

g. Stability Study

The optimized mucoadhesive oral film was stored under controlled temperature and humidity conditions at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\%$ RH for a specified period. At predetermined intervals, samples were evaluated for appearance, thickness, folding endurance, surface pH and disintegration time to assess formulation stability²².

h. Statistical analysis

The response surface methodology (RSM) was used to optimize the formulation of mucoadhesive oral films containing clove oil and green tea extract. It evaluated the effects of selected response. The response surface plots illustrated the interaction between independent variables and responses, helping identify the optimized formulation with desirable physicochemical, mechanical, mucoadhesive, and drug-release characteristics for effective dental caries management¹⁸.

RESULT

Phytochemical screening

The green tea extract shows presence of carbohydrate, alkaloids, glycosides, saponins, tannins, amino acids, phenols, flavonoids, etc. whereas the clove oil shows presence of flavonoids.

Total Phenolic Content

The green extract showed a progressive increase in total phenolic content with increasing concentration, ranging from **4.54 mg GAE/g extract at 10 µg/mL** to **78.10 mg GAE/g extract at 100 µg/mL**. The calibration curve exhibited good linearity ($R^2 = 0.9777$), confirming the suitability of the analytical method for quantification. The findings confirm that the green extract is a valuable natural source of phenolic compounds with potential applications in herbal medicinal formulations.

Total Flavonoid Content

The total flavonoid content of the green extract was determined using the aluminium chloride colorimetric method with quercetin as the reference standard. The quercetin calibration curve exhibited excellent linearity ($R^2 = 0.9999$), indicating the reliability of the analytical method for flavonoid quantification.

The green extract showed a concentration-dependent increase in absorbance and flavonoid content, ranging from 2.43 mg QE/g extract at 10 µg/mL to 100 mg QE/g extract at 100 µg/mL. Compared with the standard quercetin, the green extract demonstrated a similar linear increase in absorbance with increasing concentration, indicating the presence of appreciable amounts of flavonoids.

Antioxidant assay

The green tea extract demonstrated conc. Dependant DPPH radical scavenging activity throughout the study. At lower conc. (10-20 µg/ml), the scavenging activity (10.20-32.16%) was lower than that of ascorbic acid (27.81- 49.96%). At 40 µg/ml, the extract achieved 67.31% scavenging activity, which remained lower than the standard but indicated substantial antioxidant potential. Intensively, at higher conc. (60-100 µg/ml), the green tea extract exhibited 96.10-97.01% scavenging activity, becoming almost equivalent to ascorbic acid.

Physical examination

Table no.04 Physical examination study

Batch No.	Weight (gm)	Thickness (mm)	Folding endurance	Surface pH	Disintegration time (min)	Swelling index
F1	1.34	0.02	>300	6.44	20.54	23.77
F2	1.20	0.01	>300	6.30	18.1	14.45
F3	1.21	0.02	>300	7.03	8.2	12.91
F4	1.25	0.02	>300	7.13	15.35	20.23
F5	2.066	0.02	>300	6.32	15.8	17.16
F6	1.21	0.02	>300	7.03	8.2	12.91

Mucoadhesive Strength

The mucoadhesive properties of **mucoadhesive oral film** were evaluated using **fresh porcine buccal mucosal tissue** as the biological substrate. The formulation exhibited a **peak detachment force of 0.268 ± 0.02 N**, indicating a satisfactory adhesive interaction between the formulation and the mucosal surface. The **work of adhesion** was measured as **8.74 ± 0.42 N·mm**, suggesting that a considerable amount of energy was required to separate the formulation from the porcine tissue, reflecting sustained adhesive performance. Furthermore, the **detachment distance of 1.86 ± 0.08 mm** indicates that the formulation-maintained contact with the mucosal surface over an appreciable displacement before complete separation, demonstrating good elasticity and cohesive characteristics of the adhesive matrix. Collectively, these findings confirm that mucoadhesive oral film **possesses mucoadhesive strength, Antimicrobial efficacy**

which is expected to enhance mucosal residence time, improve localized drug retention, and potentially increase the bioavailability of the incorporated therapeutic agent for buccal or transmucosal drug delivery applications.

Drug Content Uniformity

Content uniformity was checked using three randomly selected 2×2 cm² film samples from different areas. Their green tea extract concentrations were 0.1122, 0.1149, and 0.1118, with a mean of 0.1130 ± 0.00168 . The %RSD was 1.49%, showing very little variation between samples. This indicates that the extract was evenly distributed throughout the film, likely due to proper mixing and uniform drying. The results meet the acceptable %RSD limit of below 2%, confirming good content uniformity.

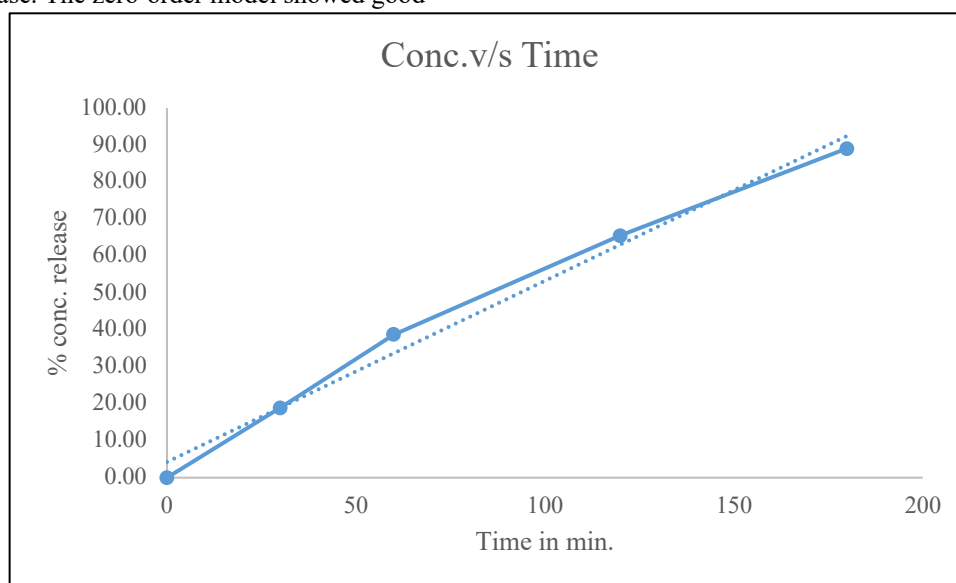
Table no.06 Antibacterial activity of Film

Sr. no.	Sample code	Zone of inhibition (mm)	
		<i>Streptococcus Mutans</i>	<i>Lactobacillus acidophilus</i>
1.	Control	00	00
2.	Standard (Streptomycin)	24	25
3.	Mucoadhesive oral film	16	14

In-Vitro Release Test

The in vitro drug release study was carried out using a Franz diffusion cell with phosphate buffer (pH 6.8) at $37 \pm 0.5^\circ\text{C}$. Samples were withdrawn at different time intervals up to 300 minutes and checked at 278 nm. The film showed slow and sustained drug release. The zero-order model showed good

linearity for green tea extract ($R^2 = 0.9951$) and clove oil ($R^2 = 0.9359$). The first-order model gave an R^2 of 0.9235. The Korsmeyer–Peppas model showed a good fit ($R^2 = 0.9709$), suggesting diffusion with anomalous transport. The Higuchi model also fitted well for green tea extract ($R^2 = 0.947$), while clove oil showed moderate fitting ($R^2 = 0.789$).

**Fig.no.01 Conc. V/S Time plot of Green tea extract release****Table no. 02 Kinetics data of Formulation FZ**

Formulation parameter	In-vitro release of Green Tea extract
Zero order model (R^2)	0.9951
First order model (R^2)	0.9973
Higuchi model (R^2)	0.947
Korsmeyers - Peppas model (R^2)	0.9966

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Statistical analysis

The 3D surface plots show how HPMC (X1) and Carbopol (X2) affect the swelling index and disintegration time of the films. Swelling increased with higher amounts of HPMC and Carbopol, especially when both were used at higher levels. The disintegration time was lower around the middle range of polymer concentrations and increased at both lower and higher levels. The curved surfaces suggest that both polymers

work together and affect these properties. Overall, the plots show that changing the amounts of HPMC and Carbopol can change the swelling and disintegration of the prepared buccal films.

$$Y1 \text{ (Swelling Index)} = -1.49853 + 6.73071 * \text{HPMC} + 28.5226 * \text{Carbopol} + -19.4436 * \text{HPMC} * \text{Carbopol} + -9.60497 * \text{HPMC}^2 + -206.063 * \text{Carbopol}^2$$

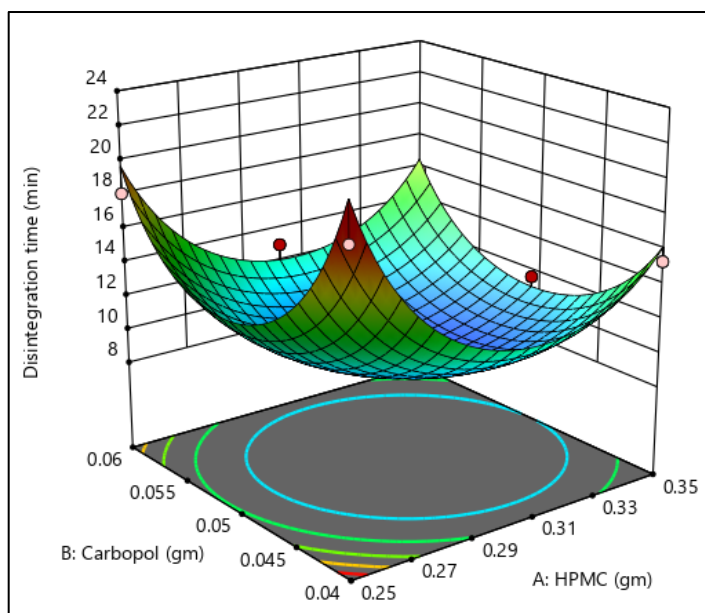


Fig.no.03 Response surface 3D graph of Swelling Index

$$Y2 \text{ (Disintegration time)} = -3.11436 + 13.2014 * \text{HPMC} + 56.7981 * \text{Carbopol} + -9.03167 * \text{HPMC} * \text{Carbopol} + -20.7582 * \text{HPMC}^2 + -537.414 * \text{Carbopol}^2$$

Fig.no.04 Response surface 3D graph of Disintegration time

Stability Data

The optimized film remained unchanged throughout the study time period. No significant change in color, appearance,

smoothness and other parameters. The thickness of the film showed slightly change due to evaporation of water content.

Table no.08 Stability data of 3 months

Parameter	Initial	1 month	2 month	3 month
Appearance	Light brown, smooth	Light brown, smooth	Light brown, smooth	Light brown, smooth
Surface pH	7.03	7.05	7.06	7.07
Thickness (mm)	0.020	0.020	0.019	0.019
Disintegration time (min)	8.2 ± 0.6	8.9 ± 0.7	8.9 ± 0.7	9.0 ± 0.6
Swelling index	12.91	12.89	12.83	12.83

DISCUSSION

The prepared mucoadhesive oral film containing green tea extract and clove oil showed satisfactory formulation characteristics. The films were flexible, had acceptable surface pH and showed good folding endurance. The optimized

formulation showed uniform drug content and adequate mucoadhesive strength, which may help the film remain in contact with the oral mucosa for a relative longer period. The formulation showed antibacterial activity against *Streptococcus mutans* and *Lactobacillus acidophilus*. Design Expert analysis showed that HPMC and Carbopol concentrations influenced

Formulation And Evaluation Of Mucoadhesive Oral Film Containing Clove Oil And Green Tea Extract For Dental Caries swelling and disintegration time. The optimized formulation also remained physically stable during three months of storage.

CONCLUSION

The study successfully developed a mucoadhesive oral film containing green tea extract and clove oil for dental caries. The prepared films showed good flexibility, suitable surface pH, satisfactory folding endurance and uniform drug content. The optimized formulation showed good mucoadhesion, which can help the film remain at the site of application for a longer time. Antibacterial activity was observed against *Streptococcus mutans* and *Lactobacillus acidophilus*. The film also provided gradual release of the active ingredients. HPMC and Carbopol affected the swelling and disintegration properties of the films. The optimized formulation remained stable during the three-month stability study, indicating its potential for localized oral application.

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