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Research

Development and Evaluation of Baicalein & Berberine Containing Dental Gel for Human Periodontal Disorder Management

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Abstract

Periodontal diseases are primarily caused by infections and inflammation affecting the gums and the bone structure that support the teeth. These conditions are highly prevalent across the globe, impacting individuals of all ages, from children to adults. In the context of oral hygiene, dental gels play a vital role by delivering therapeutic agents directly to the oral cavity, thereby helping to manage and prevent such diseases. This study focused on the development and optimization of a dental gel using Carbopol 934 as the gelling agent. The aim was to formulate a dental gel containing the active compounds baicalein and berberine, known for their antimicrobial properties. Various concentrations of carbomer were utilized to develop different gel formulations, which were then subjected to a comprehensive evaluation of their physicochemical properties. These properties included pH, color, clarity, viscosity, consistency, homogeneity, and spreadability. Through thorough analysis, the optimal gel formulation was identified based on its superior physicochemical characteristics. This optimized formulation was subsequently tested for antimicrobial activity using the agar disc diffusion method. The results from this testing were promising, indicating that the dental gel exhibited significant antimicrobial activity against the bacterial strains used in the study. Overall, the study successfully demonstrated that the dental gel, formulated with baicalein and berberine and optimized with Carbopol 934, has potential as an effective antimicrobial agent in the treatment and prevention of periodontal diseases.

Keywords: Periodontal diseases, Dental Gel, Baicalein, Berberine and Carbopol 934.

INTRODUCTION

Periodontal diseases represent a significant global health burden, affecting the supportive tissues surrounding teeth and leading to substantial morbidity if left untreated. Traditional treatments often face

challenges such as patient compliance and antimicrobial resistance. Recently, natural compounds like baicalein and berberine have gained attention for their potent anti-inflammatory and antimicrobial properties [1]. Baicalein, derived from Scutellaria baicalensis, and berberine, from Berberis species, have shown promising results in periodontal therapy due to their ability to inhibit inflammatory mediators and pathogenic bacteria [2]. Incorporating these compounds into a dental gel formulation could provide a novel approach for managing periodontal disorders effectively. This study aims to develop and evaluate such a baicalein and berberine-containing dental gel, assessing its therapeutic efficacy through invitro antimicrobial evaluations.

MATERIALS AND METHOD

Materials

Baicalein and berberine were purchased from TCI Ltd, India. Carbomer 934 and Triethanolamine were purchased from Qualikems Finechem Pvt Ltd; Propanediol was purchased from Nice chemicals Pvt. Ltd, Mumbai. All other reagents were analytical grade.

Development of UV spectroscopic methods for quantitative analysis

Preparation of standard solution and Calibration curve of baicalein

An accurately weighed quantity (1mg) of baicalein was dissolved in distilled water and made up to 200ml with distilled water in a volumetric flask and designed as Stock solution-1 Into series of the volumetric flasks (100ml), aliquots of the sample (25, 50, 75 & 100 ml) were taken from stock solution-1 and made up to 100ml with distilled water to obtain 1.25, 2.5, 3.75, 5 µg/ml concentrations respectively.. The absorbance values at the λ_{max} 274 nm obtained in each case was plotted against concentration to obtain a calibration curve.

Preparation of standard solution and Calibration curve of berberine

An accurately weighed quantity (2mg) of baicalein was dissolved in distilled water and made up to 50ml with distilled water in a volumetric flask and designed as Stock solution-1 Into series of the volumetric flasks (10ml), aliquots of the sample (1.25, 2.5, 5, &10 ml) were taken from stock solution-1 and made up to 10ml with distilled water to obtain 5, 10, 20, & 40µg/ml concentrations respectively The absorbance values at the λ_{max} 344 nm obtained in each case was plotted against concentration to obtain a calibration curve. The linear correlation between these concentrations(x- axis) and absorbance (y-axis) were graphically presented and slope (b), intercept (a), and correlation coefficient (r^2) were calculated for the linear equation (y=bx+a).

Method

Preparation of Dental Gel

Dental gels were prepared by soaking carbomer 934 in 10 ml of water and adjusting the pH to 6.4 using triethanolamine to form the gel [3]. A weighed amount of propyl paraben was added to the water prior to the addition of carbomer 934. In a separate beaker, the required quantity of propanediol was taken, and the accurately measured amounts of baicalein and berberine, corresponding to their minimum inhibitory concentrations (MIC), were incorporated into it [4]. This mixture was then added to the beaker containing the carbomer with continuous stirring. A sweetener was also added to the polymer dispersion and mixed until a homogeneous mixture was obtained [5]. Distilled water was used to make up the volume, and the mixture was stirred vigorously. All the prepared dental gels were then subjected to evaluation to select the best formulation [6]. The composition of different gel formulations is shown in Table 1.

Table 1: Exhibits the formulation for preparation of dental gel

Ingredients	BBDG1	BBDG2	BBDG3	EBEDG4	BBDG5
Baicalein(mg)	1	1	1	1	1
Berberine(mg)	1	1	1	1	1
Carbomer (mg)	200	400	600	800	1000
Triethanolamine*	qs	qs	qs	qs	qs
Propanediol (ml)	10	10	10	10	10
Propyl paraben (g)	0.18	0.18	0.18	0.18	0.18
Aspartame (g)	0.4	0.4	0.4	0.4	0.4
Distilled water*	q.s	q.s	q.s	q.s	q.s

**qs- Quantity Sufficient to bring the gel to pH 6.8*

Compatibility study (FTIR)

FTIR is recommended as a simple technique for the detection of interactions between the API and the excipients or between different APIs. FTIR study was carried out to discover baicalein and berberine and its compatibility with Carbopol 934. The samples were prepared by grinding the dry specimens with KBr and pressing them to form disks. These analyses were performed within the range of 400–4000 cm^{-1}.

Characterization of Prepared Gel: Evaluation of gel formulation

Physical appearance

Color: The color of the formulation was checked out against a white background.

Consistency: The consistency was checked by applying on skin.

Greasiness: The greasiness was studied by the applicationon to the skin.

Determination of pH: The pH of each gel was measured using a digital pH meter (model 111, ESICO, India) by immersing the electrode completely into the gel system [7].

Determination of Viscosity: Viscosity measurements of the formulated gels were conducted using a Brookfield viscometer (model DV1, Brookfield Engineering Laboratories, Inc, USA) equipped with spindle no. 7, operating at a spindle speed of 60 rpm and maintained at 25°C. The corresponding dial reading on the viscometer was recorded for each gel formulation [8].

Determination of Extrudability: Extrudability was assessed by filling a tube with the gel, which had a 5 mm opening at the tip. The amount of gel extruded through the tip under a standardized pressure was quantified and recorded [9].

Determination of Homogeneity: The homogeneity of each gel formulation was evaluated visually after the gels had solidified in their respective containers. They were inspected for uniformity in appearance and the absence of any visible aggregates [10].

Determination of Spreadability: Spreadability was determined using a modified wooden block and glass slide apparatus. The apparatus consisted of a fixed glass slide and a movable glass slide connected to a pan via a string and pulley system. A measured quantity of gel was placed on the fixed glass slide, and after placing the movable glass slide on top to sandwich the gel, a weight of 50 grams was added to the pan. The time taken for the slides to separate under the applied weight was recorded. Spreadability (S) was calculated using the formula:

$S = M.L/T$

where S is the spreadability in grams·cm/second, M is the mass in grams, L is the length of movement in cm, and T is the time in seconds[11].

Fig 1: Modified wooden block and glass slide apparatus

Determination of Drug Content

An accurately weighed quantity of 1 g dental gel was dissolved in 100 ml of phosphate buffer (pH 6.8). The solutions were shaken for 2 hours and then allowed to stand for 5 hours to ensure complete dissolution of the drugs from the gel. After dissolution, the solutions were filtered through a 0.45-µm nylon filter. The drug content was estimated using a UV spectrophotometer (Shimadzu 1900i) at 274 nm for baicalein and 422 nm for berberine, calculated from the calibration data [12].

Determination of Antimicrobial Activity

The antibacterial activity of the gel containing baicalein and berberine was evaluated using the agar cup plate method. The optimized formulation was aseptically placed in agar plates that had been inoculated with bacterial culture. The plates were left at room temperature for 30 minutes before being incubated at 37°C for 48 hours. Tetracycline, a broad-spectrum antibiotic, was used as a positive control for comparison. After 24-48 hours of incubation, the plates were examined for zones of inhibition. The antimicrobial activity was assessed by measuring the diameter of the inhibition zones in millimeters [13].

RESULTS AND DISCUSSION

Development of a UV Spectrometric Method for Measuring Baicalein and Berberine in Drug Formulations

A UV spectrometric method was developed to accurately measure the concentrations of baicalein and berberine in drug formulations. Calibration curves were established for both drugs, with the maximum absorbance for baicalein observed at 274 nm and for berberine at 344 nm. The method demonstrated linearity within the concentration ranges of 2-8 µg/ml for baicalein and 2-40 µg/ml for berberine, with correlation coefficients (r^2) of 0.996 and 0.999, respectively. This validated method was subsequently employed to quantify the amounts of baicalein and berberine in various formulations [5& 8].

Infrared Spectroscopic Analysis

The Infrared (IR) spectra of baicalein, berberine, and their physical mixture with carbomer 934 are presented in Figures 2-4. The baicalein spectrum exhibited characteristic peaks at 3484 cm^{-1}, corresponding to O-H stretching, and at 1658 cm^{-1}, corresponding to C=O stretching [7]. The FTIR spectrum of berberine showed a characteristic peak for C-H stretching at 2844 cm^{-1}, while peaks for C=C and C=N stretching were observed at 1597.81 cm^{-1}. The deformation in C-H was found in the range of 1362-1388 cm^{-1}, and C-O stretching was observed at 1034 cm^{-1} [4]. The physical mixture contained the peaks of both drugs, indicating no chemical interaction between the drugs and the excipients [10].

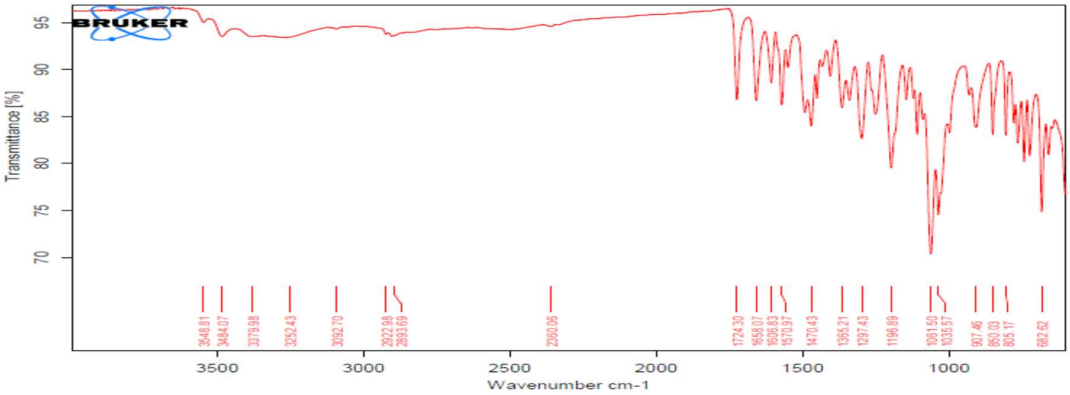

Fig 2: FTIR Spectra of baicalein

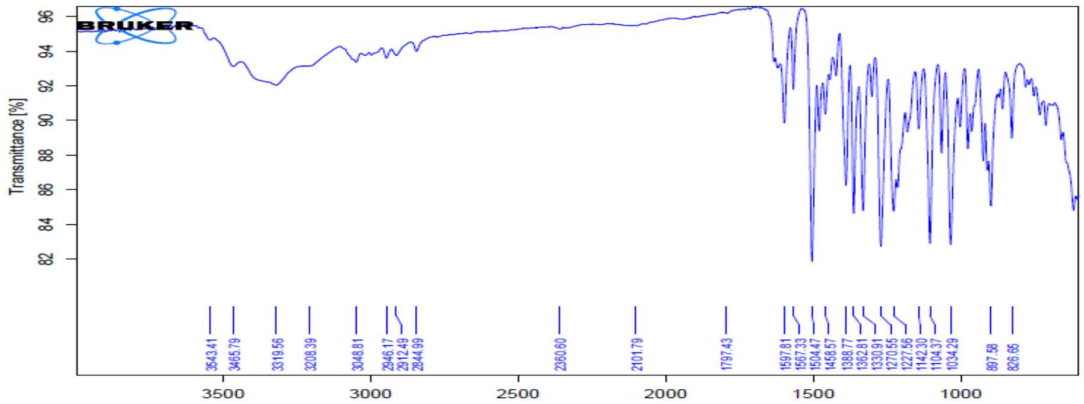

Fig 3: FTIR Spectra of berberine

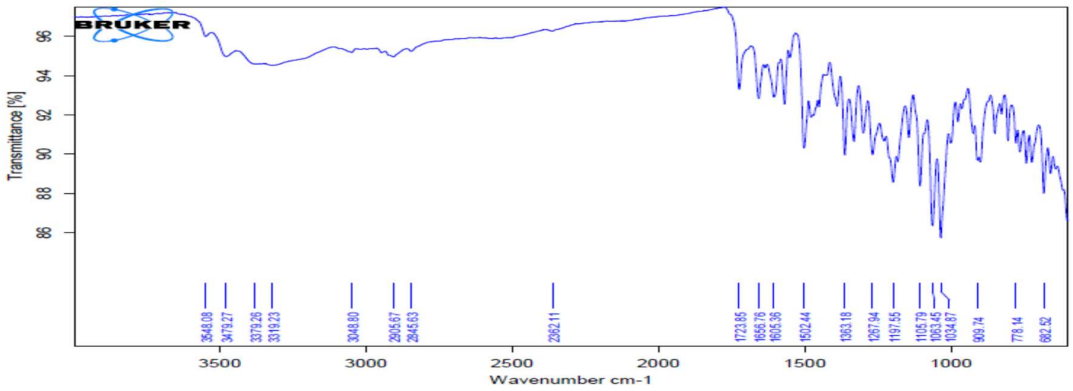

Fig 4: FTIR Spectra of physical mixture of baicalein, berberine and carbomer934

Preparation of Dental Gel Containing Baicalein and Berberine

The dental gel containing baicalein and berberine was formulated using varying concentrations of carbomer 934 and triethanolamine. The resulting gels exhibited good consistency and non-greasy texture with no specific odor.

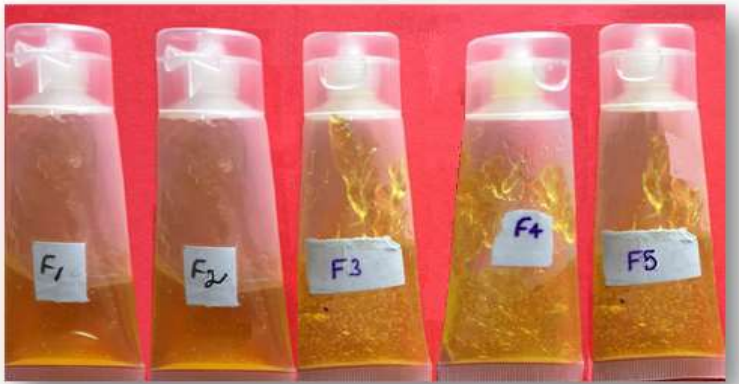

Fig 5: Prepared dental gel (F1-F5)

Physico-Chemical Characterization of Dental Gel

All formulations exhibited a pH range of 6.3-6.5, which is compatible with buccal pH and unlikely to cause irritation when applied as a dental gel. The spreadability ranged from 3.1 to 1.02, indicating that higher concentrations of carbomer result in a thicker gel. Similar findings were observed in the extrudability studies, where gel consistency increased with higher carbomer concentrations. Additionally, the viscosity of the gel also increased with the concentration of carbomer. The results were presented in table2.

The drug content study showed values between 98.4% and 99.8% for baicalein and between 98.3% and 99.7% for berberine, as presented in table 3.

Table 2: Physiochemical characterization of prepared dental gels

Formulation code	pH	Viscosity (cps)	Spreadability (g-cm/sec)	Extrudability (g/g of gel)	Homogeneity
F1	6.4.	40121	3.10±0.31	36.2±2.9	Very Good
F2	6.4	41602	2.86±0.23	40.3±1.9	Very Good
F3	6.5	42426	2.56±0.82	48.3±3.3	Good
F4	6.3	434123	1.47±0.56	54.9±1.0	Good
F5	6.4	45412	1.02±0.10	66.2±0.5	Good

Table 3: % of drug content of prepared dental gels

Formulation code	Baicalein (%) ± SD	Berberine (%) ± SD
F1	99.3±0.26	98.6±0.40
F2	99.1±0.80	98.3±0.90
F3	98.4±1.10	99.7±0.37
F4	98.6±0.75	98.8±0.85
F5	99.8±0.57	99.6±0.40

Antimicrobial Efficacy Studies of Dental Gel

The dental gel formulation F3, which contains baicalein and berberine, exhibited superior physicochemical properties and higher drug content compared to other formulations (refer to Table 2 and 3). Consequently, this formulation was selected for further antimicrobial studies.

The results of these antimicrobial studies indicated that the F3 dental gel formulation demonstrated the maximum zone of inhibition (Table 4, Figures 7-9). The diameter of the zone of inhibition produced by the F3 formulation was comparable to that of the pure drugs. This confirms that incorporating the drugs into the gel base does not reduce their antibacterial activity.

Table 4: Antimicrobial activity (ZOI) of optimized dental gel formulation

Microorganism	Zone of Inhibition (mm) Formulation (F3)	Zone of Inhibition (mm) Tetracycline	Zone of Inhibition (mm)	
			Baicalein	Berberine
Candida Albicans	18±2	20±2	15±1	16±2
Escherichia coli	19±3	21±1	13±1	12±1
Bacillus subtilis	23±2	25±3	18±2	15±2

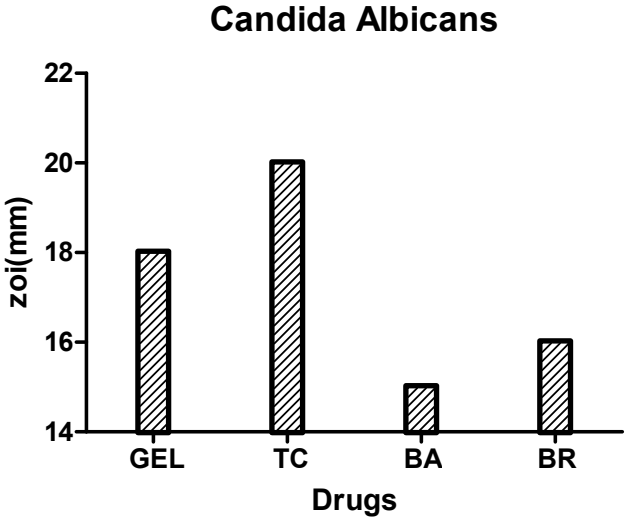

Fig 6: Graphical presentation the effects of berberine and baicalein, alone and in combination (F3) against Candida Albicans

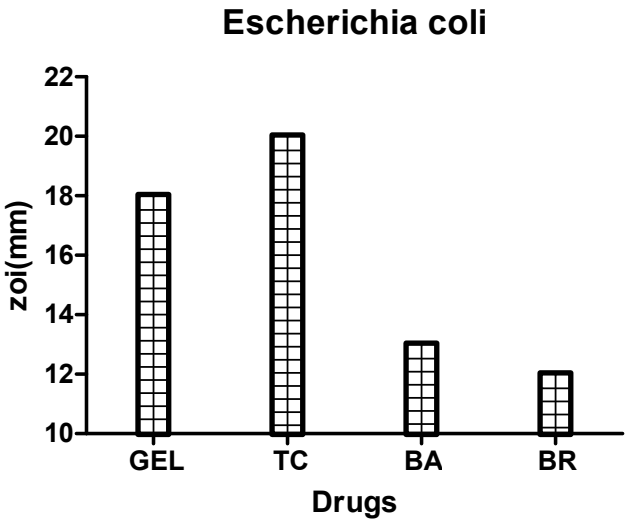

Fig 7: Graphical presentation the effects of berberine and baicalein, alone and in combination (F3) against *Escherichia coli*

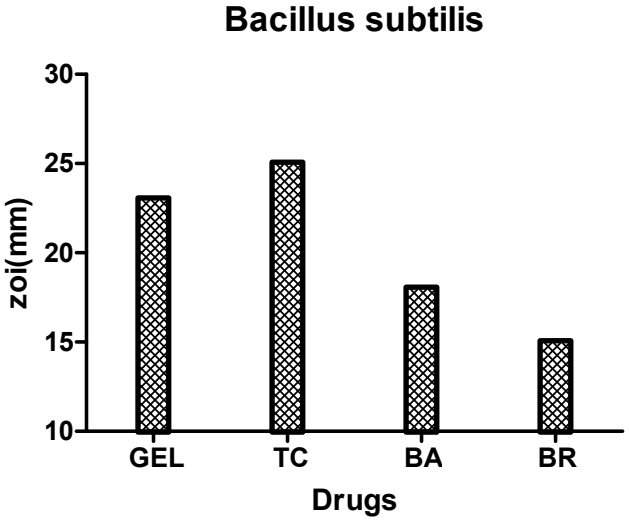

Fig 8: Graphical presentations the effects of berberine and baicalein, alone and in combination (F3) against *Bacillus subtilis*

The dental gel formulation (F3) exhibited antimicrobial activity against three microbes: *Escherichia coli, Bacillus subtilis*, and *Candida albicans*, as assessed by measuring the inhibition zone diameters (mm). Specifically, the inhibition zones were measured as 18±2 mm for *Candida albicans,* 19±3 mm for *Escherichia coli*, and 23±2 mm for *Bacillus subtilis*. In contrast, when the bioactive compounds baicalein and berberine were tested individually, their inhibition zones against *Candida albicans*, *Escherichia coli*, and *Bacillus subtilis* were 15±1 mm & 16±2 mm, 13±1 mm & 12±1 mm, and 18±2 mm & 15±2 mm, respectively. These findings indicate that baicalein and berberine alone exhibit lower efficacy against all three microbes compared to the dental gel, which demonstrates larger inhibition zones. This enhanced effectiveness of the dental gel formulation may be attributed to synergistic effects resulting from the combination of both compounds.

CONCLUSION

A dental gel containing baicalein and berberine was successfully developed, demonstrating promising potential for managing periodontal diseases. The gel exhibited good consistency, compatibility with buccal pH, and preserved the antimicrobial potency of the active compounds. Notably, the F3 variant showed superior

properties and effective antibacterial activity, suggesting its suitability for commercial dental gel applications. Further studies are recommended to validate its use before commercialization.

Declaration of Interest

The authors declare no conflicts of interest related to this work. The responsibility for the content and writing of this paper lies solely with the authors.

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