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Research Article

Unlocking traditional remedy: Gulkand-enhanced mucoadhesive gel for canker sore relief

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Abstract: This study focuses on the development and evaluation of mucoadhesive gel formulations containing different Gulkand and its extract with a specific emphasis on their potential for managing oral health conditions, including the discomforting issue of mouth ulcers. The formulations underwent a thorough analysis, encompassing the assessment of phenolic compounds responsible for antioxidant activity, in addition to comprehensive physical, chemical, and pharmacological evaluations to determine their suitability for commercial utilization. Mouth ulcers are a prevalent oral health concern that can cause significant discomfort and inconvenience. In this study, Gulkand extract exhibited remarkable characteristics with its high phenolic content and robust antioxidant activity. It demonstrated rapid drug release, positioning it as a promising new for addressing the immediate relief needs of individuals suffering from mouth ulcers. Conversely, gel formulation showcased a sustained release profile suggesting the potential for longer therapeutic benefits, presents an intriguing option for oral health applications, capitalizing on the antiulcer properties associated with liquorice. An innovative aspect of this study is the compatibility assessment, which employed antioxidant and phenolic content analysis to verify the harmonious interaction between herbal constituents and excipients. This approach introduces novel perspective on compatibility testing, particularly critical for formulations designed to alleviate oral discomfort effectively. Furthermore, stability studies are warranted to be evaluated prior to their potential for commercialization. The present study revealed the promising potential of the prepared mucoadhesive gels in managing not only mouth ulcers but also a spectrum of oral health conditions, holding broader implications for advancements in the field of medicine.

1. INTRODUCTION

People around the globe suffer from oral ulcers once or many times during their lifetime. Mouth ulcers are the lesions that develop within the tender tissue bordering the lips, palate, inner cheek, or gums (Burley *et al.*, 2021). Canker lesions are another indication of their use. Mouth ulcers can affect people of all ages, typically more of an annoyance than a significant scientific challenge (Rai *et al.*, 2022). Even though it is no longer harmful, but are found to be linked to various diseases and conditions,including diabetes, immune disorders, inflammatory bowel

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disease, celiac disease, HIV/AIDS, and Behçet's disease (Abed *et al*., 2019; Baarah *et al.*, 2017; Seoudi *et al*., 2015). Mouth ulcers are easy to detect, usually developing on the lips, roof of the mouth, or inner cheeks of younger individuals (Ussher *et al*., 2003). Their centers are typically white, yellow, or gray, and one or more may be present in the pharynx (Dudding *et al*., 2019). Symptoms include swelling around the ulcer, prolonged tenderness when brushing the teeth, and pain that worsens after consuming highly spiced, salted, or acidic foods (Ballen *et al*., 2016; Qu *et al*., 2022). The exact cause of the buccal ulcer remains uncertain, with multiple factors contributing to their formation, such as allergic reactions to specific microbes, vitamin deficiency, using abrasive toothpaste, consuming an abundance of acidic foods, hormonal fluctuations, stress, and lack of sleep (Zhou *et al*., 2022; Orcina & Santos, 2021; Ussher *et al*., 2003).

1.1. Types of Mouth Ulcers

Based on size and quantity, mouth ulcers are classified into:

• Minor ulcers: Diameter of 2.8 mm, typically resolving in 10–13 days (Dudding *et al*., 2019).

• Major ulcers: Larger, deeper, may leave a scar, and may take few weeks to heal (Ballen *et al.*, 2016).

• Herpetiform ulcers: A collection of several tiny and, pinhead-sized lesions (Dudding *et al.*, 2019; Amin & Marouf, 2022).

1.2. Treatment and Side Effects of Mouth Ulcers

Common treatments includes application of antiseptic, local anesthetic gels, steroid ointments, and medicinal mouth ulcer rinses (Fan *et al.*, 2022). Drugs that reduces the oxidative stress by reducing the level of free radicals also plays an important in the treatment of mouth ulcers. Oral health, especially mouth ulcers, requires antioxidant activity. Studies link periodontal disease to reduced salivary antioxidant capacity, leading to increased oral cavity oxidative damage (Bhattacharya *et al*.,2014). Antioxidants known for their anti-ulcer properties, and their chemical composition have also been studied for gastric ulcers (Danisman *et al*.,2023). Antioxidants like beta carotene, selenium oxide, and zinc sulfate have been studied for treating oral submucous fibrosis, demonstrating their potential benefits (Rao *et al.*,2020). However, these may result in side effects such as inadequacy, immunosuppressant effects, osteoporosis, hyperglycemia, and gastrointestinal disturbances (Mittal *et al.*, 2023; Ballen *et al.*, 2016). Commercial formulations with synthetic and semi-synthetic active components may cause local discomfort and stains due to high alcohol content and chemical compounds (Mittal *et al*., 2023). The use of plant-based medicines is gaining significance globally due to increased patient compliance and demand (Proestos *et al.*, 2013). Herbal remedies of mouth ulcer includes chewing, consumption or application of *Basella alba, Glycyrrhiza glabra*, *Psidium guajava*, *Aloe barbadensis*, *Capsicum* sp., *Carica papaya*, *Curcuma longa*, various species of *Rosa*, *Bathinda variegate*, and *Hibiscus rosa sinensis*, are considered as safer alternatives (Çiçek *et al*., 2022; Proestos *et al*., 2013; Kumar *et al.*, 2009). In various parts of India, it was observed that Gulkand is generally applied on the mouth ulcer to treat. Gulkand, a sweet preserve made from rose petals, involves the use of rose petals from several rose species, with *Rosa damascena* being a key component (Çiçek *et al*., 2022; Fascella *et al*., 2022; Yap *et al*., 2011). Gulkand, also known as rose petal jam, is derived from the Persian words gul (rose) and qand (sweet/sugar) and is served at room temperature in the Indian subcontinent (Bhatt *et al*., 2000). Damask rose, with its powerful scent, is frequently used in the manufacture of Gulkand recipes and blossoms only twice a year, making it exclusive and sugars are generally used as a sweetening agents in the preparation of Gulkand (Çiçek *et al.*, 2022). The ideal roses employed for preparing gulkand are *Rosa centifolia* and *Rosa damascena* (Proestos *et al.*, 2013; Hajizadeh *et al*., 2023).

Hence, the primary objective of the present study was to prepare the Gulkand, and its formulations containing sugars and *Glycyrrhiza glabra* as an alternate to sugar and evaluating

their antioxidant efficacy. Secondarily an effort was also made to develop a simple method to evaluate compatibility of Gulkand and the excipients using UV and FTIR spectroscopy. Additionally, diffusion study was also conducted to confirm the amount of antioxidants available for the therapeutical efficacy.

2. MATERIAL and METHODS

2.1. Materials

In the preparation of Gulkand, a traditional confectionary delicacy, a blend of natural ingredients was employed, sourced meticulously from local markets. The petals of *Rosa damascena*, roots of *Glycyrrhiza glabra* (liquorice), and rock sugar played pivotal roles in crafting the distinctive flavor profile of the preparation. Alongside these botanical elements, a range of solvents and chemicals, including alcohol, methanol, DPPH, sodium carbonate, Folin-Ciocalteu reagent, hydrochloric acid, and vanillin were also employed in the present study. Pharmaceutical excipients such as carboxymethyl cellulose, polyethylene glycol 4000, methyl paraben, propyl paraben, and triethanolamine were carefully selected and sourced from reputed suppliers like E-Merck and Fischer. The use of various instrumental techniques, such as a freeze dryer, sonicator, UV spectrometer, Fourier-transform infrared spectroscopy (FTIR), and a rotary evaporator, ensured precision in the preparation process. This comprehensive combination of plant materials, solvents, chemicals, and instruments establishes a robust foundation for a detailed investigation into the properties and potential applications of the developed buccal mucoadhesive gel.

2.2. Methods

2.2.1. *Preparation of different types of Gulkand using different sweetening agents*

2.2.1.1. Method 1 Rose petal + Rock sugar (R+R). *Rosa damascena* fresh rose petals that have been picked are properly cleansed in clean flowing water and RO water before being dried in the shade. After drying, rose petals are crushed and combined with rock sugar in a 2:1 ratio in a glass jar with a wide mouth that is clean, dry, and airtight for a period of five days while exposed to the sun. During this period, the sugar totally melted and was thoroughly blended with the rose petals that makes the rose petals' nutrients intact.

2.2.1.2. Method 2 Rose petal + Liquorice (R+L). The collection and preparation of rose petals was carried out as the procedure given in the section 2.2.1.1. Exactly to the 50 g of dried liquorice (*Glycyrrhiza glabra*) powder, 500 mL of water was added, and then it was boiled at 100°C to produce the liquid extract. A magnetic stirrer was used to stir continuously for 30 minutes. In a mixing dish, 200 g of crushed rose petals were combined with 200 mL of the aforementioned liquorice extract and along with some preservatives. The prepared mixture was placed in a clean, dry, and airtight glass jar with a wide mouth till the formation of Gulkand.

2.2.1.3. Method 3 Rose petal +Liquorice prepared on frying pan (R+L, pan). In this method, liquorice Gulkand was prepared by using a frying pan. Hundred grams of the rose petals were gathered and added to the pan. In a clean skillet, 100 mL of the liquorice extract prepared in step 2 was added and cooked until it turned to brown colour. This procedure was aimed to maintain the nutrients found in rose petals. The resulting mixture was stored in a clean, dry, and airtight glass jar with a wide opening.

A pilot study was performed to determine the stability of the prepared samples prior to the use of methods 2 and 3.

2.2.2. *Evaluation of gulkand*

The prepared Gulkands' colour, flavour, stability, and consistency were assessed at regular intervals.

2.2.3. *Drying, powdering and extraction of gulkand composed of liquorice*

2.2.3.1. Drying. The aforementioned preparations (R+R, R+L, and R+L pan) were dried in a lyophilizer after few days. Below given steps are followed to dry the prepared samples:
Step 1: Samples are maintained in a beaker with aluminium foil covering them.
Step 2: Samples are stored in the primary drying chamber for 11 hours and the secondary drying chamber for 12 hours after being pre-frozen in the freezer.

2.2.3.2. Powdering. While powdering the freeze dried samples, a temperature of 35° C was maintained throughout the process in order to preserve the bioactive phytoconstituents present in the prepared samples. To keep the samples fresh for a long period, the powdered samples are placed in a centrifuge tube, sealed with parafilm, and preserved there.

2.2.3.3. Extraction. Only sample 2 (R+L) underwent extraction since it had higher antioxidant activity than the other samples.

Preparation of extract of R+L sample: *Rosa damascena* petals powdered gulkand and 60g of liquorice were placed in a conical flask and extracted for 1 hour using the sonication process with 80% of 1200 mL alcohol (300 mL X 4 times). The extracted solvent was transferred into a rotary evaporator operated at 50° C. The dried extract was collected after the concentrated viscous extract was once again maintained in a water bath at a temperature of 50^{o} C.

2.2.4. *Determination of antioxidant activity*

The DPPH test measures the antioxidant capacity of the herbal extract, as well as its composition, standards as well as also employed for the first time to study the compatibility of samples. DPPH was dissolved in methanol to create the DPPH reagent, which was then further diluted to get the final concentration of 0.4 mm DPPH. Gallic acid was utilised as a positive control while various concentrations of extract, powder, petals, and formulations were made by dissolving in methanol. One mililitre of the DPPH reagent was added to the various sample concentrations used in the test, vortexed, and left in the dark for 30 minutes. A UV visible spectrophotometer was used to evaluate the reduction in absorbance caused by the scavenging of the DPPH free radical at a wavelength of 517 nm (Proestos *et al*., 2013).

The antioxidant activity in terms of percentage inhibition was determined using equation

$$\% \text{ inhibition} = (Ac\text{-}As/Ac) \times 100$$

Where, As an Ac is the absorbance of the sample solution and control, respectively.

2.2.5. *Total phenolic content*

Using the Folin-Ciocalteu reagent, a complicated combination of heteropolyphosphotungstate molybdate, the total quantity of phenolic-related chemicals contained in the sample was calculated. In the presence of sodium carbonate, phenols undergo FC reagent treatment and produce a blue colour complex. The quantity of reactive phenolic chemicals in the sample directly relates to how intense the blue colour is. This approach can measure the overall polyphenolic content of the extracts, which ranges from 5 to 100% (w/w), in terms of standard gallic acid.

Procedure:

➢ The standard calibration curve was prepared using gallic acid.

➢ Folin-Ciocalteau reagent was added after sample stock solutions were created at a concentration of 10 g/mL.

➢ 1.6 mL of Na_2CO_3 (7.5%) was added as an alkaline medium after a 6 minute break.

➢ The mixture was completely vortexed before being left to incubate in the dark for a full hour.

➢Using a UV-Visible Spectrophotometer set at 765 nm, the color developed by the samples was examined, and the absorbance was recorded (Kumar *et al*., 2009).

2.2.6. *Crude drug- excipient interaction studies*

Gulkand powders of R+R, R+L, and extract of R+L are utilised to create gel formulations. Prior to formula preparation, the following procedures were used to assess compatibility:

2.2.6.1. Drug –Excipients interaction (compatibility) study by FTIR. Fourier Transform Infrared spectrometer (FTIR) analysis is performed on samples containing R+R, R+L gelling agents such as carbopol 940, sodium CMC, Chitosan, Polyethylene glycol (PEG), and preservatives such as methyl paraben and propyl paraben. The aforementioned gulkand samples and excipients were weighed, combined, and delivered to CRI (centre of research innovation), ACU, and B. G. Nagara for FTIR for the compatibility studies (Shaw *et al.*, 2017; Zou *et al.*, 2020).

2.2.6.2. Drug –Excipients interaction (compatibility) study by antioxidant activity. The DPPH technique was used to measure antioxidant activity after dissolving the weighted quantity of samples and excipients in different amounts of water.

2.2.7. *Preparation of buccal mucoadhesive gel*

Methyl and propyl paraben were accurately weighed and dissolved in water heated to 80°C. A weighed amount of sodium CMC was dissolved in water at 50°C for 30 min while being continuously stirred with a mechanical stirrer spinning at 2000 rpm. In accordance with its solubility, PEG 4000 has transformed into liquid form. With steady stirring, the weighed amount of samples and extract are dissolved in PEG 4000 solvent, and then they are added to the gel base. To create a uniform gel, constant stirring is helpful. Triethanolamine was used to adjust the pH to 7, and the mixture was carefully agitated until a clear gel was produced (Aslani *et al*., 2013). Information related to the composition of mucoadhesive gel is given in the Table 1.

Table 1. Composition of mucoadhesive gel.

Ingredients	Formulations			Uses
	R+R (p) in g	R+L (p) in g	R+L (e) in g	
Sodium CMC	1.5	1.5	1.5	Gelling agent
Methyl paraben	0.09	0.09	0.09	Preservative
Propyl paraben	0.01	0.01	0.01	Preservative
PEG 4000	6.5	6.5	6.5	Osmotic agent
R + R powder	10			Active ingredient
R + L powder		10		Active ingredient
R + L extract			1	Active ingredient

R + R (p): Rose petal and rock sugar powder, R + L (p): Rose petal and Licorice powder and R + L (e): Rose petal and Licorice extract.

2.2.8. *Physical and chemical evaluation of buccal mucoadhesive gel*

2.2.8.1. Physical appearance of gel formulations. Clearness, color, homogeneity, consistency, and the presence of particles in gel compositions were all visually evaluated. A microscope was used to check homogeneity. A small amount of gel was pushed between the thumb and index fingers in order to examine the formulations' consistency. The gel's consistency was then noted.

2.2.8.2. Determination of pH in the gel formulations. The produced gels' pH was assessed using a pH meter, which was calibrated with standard buffer solutions at pH 4 and 7 before each usage. In 10 mL of pure water, each gel formulation was precisely weighed and mixed.

The pH of the samples was recorded after the electrode had been put into the sample for ten minutes at room temperature. The pH was measured after 48 hours, one week, two weeks, and one month.

2.2.8.3. Centrifugal test. The formulation after 48 hours of preparation were transferred into centrifuge tubes and centrifuged at 2000 rpm for 60 min using a centrifuge device (Centrifuge 5430). The stability of the formulations was assessed at the times of 5, 15, and 60 min to investigate the stability of the formulations against the centrifugal force.

2.2.8.4. Drug content determination in gel formulations. After 48 hours of preparation of gel, one gram of the gel was taken and dissolved in water in a volumetric flask with a 10 mL capacity. The Folin-Ciocalteu technique and the DPPH method were carried out to assess the total phenolic content and antioxidant activity of the prepared formulation.

2.2.8.5. Determination of viscosity. The gel formulations were studied for viscosity 30 mins after the preparation at room temperature. Brookfield DV-II viscometer was used to evaluate the produced gels' viscosity at 100 rpm and 25°C using spindle number 7.

2.2.9. *Ex vivo drug release study*

In order to carry out the drug release study goat buccal mucosa membrane was purchased from a slaughterhouse in Bellur, Mandya was employed as a barrier membrane. The gels were tested using a Franz diffusion cell for drug release. Buccal mucosal membrane was mounted between the donor and receptor compartments. The diffusion cell was positioned in simulated saliva that was kept at a constant 37 °C. A magnetic bead was used to stir at 300 rpm while 50 mL of phosphate buffer (pH 7.4) was added to the receptor compartment to maintain hydrodynamics. In order to maintain the volume of the liquid medium, 5mL of the sample was removed, and the tube was then filled with 5mL of fresh medium. The material was examined in a UV spectrophotometer at a wavelength of 226 nm.

Composition of saliva: To get a saliva of pH 6.75, add phosphoric acid to 2.38 g of Na_2HPO_4, 0.19 g of KH_2PO_4, and 8.00 g of NaCl per litre of distilled water.

Composition of Phosphate buffer solution: To 393.4 mL of 0.1M sodium hydroxide, add 250 mL of 0.2M potassium dehydrogenate phosphate (Aslani *et al.*, 2013).

2.2.10. *Statistical analysis*

Statistical analyses, including One-way ANOVA, Tukey's HSD post-hoc test, Student's t-test, correlation analysis, Chi-square test, and descriptive statistics, were conducted to evaluate experimental data. These methods compared means, identified significant differences, explored relationships between variables, and assessed compatibility. By employing these statistical techniques, the study enhanced the robustness and validity of results, providing valuable insights into the effectiveness of different gulkand samples and gel formulations.

3. FINDINGS

3.1. Preparation of Different Types of Gulkand Using Different Sweetening Agents Such as Rock Sugar and Liquorice

The collected rose petals underwent a thorough cleaning before air drying for three to five days, and the the prepared gulkand was kept in an airtight container.

3.2. Evaluation of Gulkand

3.2.1. *Morphological evaluation*

Table 2 provides the results of a morphological examination of several types of Gulkand based on their flavour, consistency, texture, and particle size. The Table 2 shows that while all of the gulkand samples had the same aromatic odour, consistency, texture, and particles, they had different tastes because the gulkand prepared with liquorice solvent had a sweet and mildly sour flavour. The semisolid consistency and pungent odour of each batch of gulkand were the same.

Table 2. Morphological evaluation of different gulkand samples.

Sample	Odour	Taste	Consistency	Texture	Particles
R+R	Aromatic	Sweet & soothen	Semi solid	Thick semi solid	Broken petals found
R+L	Aromatic	Sweet & sour	Semi solid	Thick semi solid	Broken petals found
R+L pan	Aromatic	Slightly sweet	Semi solid	Thick semi solid	Broken petals found

3.3. Antioxidant Activity of Various Samples

Using the DPPH technique, the antioxidant activity of the prepared Gulkand stored at room temperature, stored in refrigerator, freeze-dried gulkand and the extract of the Gulkand was assessed and the results of the study was depicted in the Figure 1. It is evident from the Figure 1 that among prepared Gulkand, Gulkand stored at room temperature possess good antioxidant activity at a concentration of 2.5 mg/mL, and the activity was increased after storing in refrigerator and after freeze-drying. Among the different Gulkand extracts, Gulkand of R + L freeze dried powder extract at the concentration of 2.5 ug showed significant antioxidant activity followed by R + L Gulkand, R + R Gulkand and R + L pan Gulkand at the concentration of 2.5 mg/mL. However, the activity was found to significantly lesser than gallic acid but significantly higher than ascorbic acid at the same concentration.

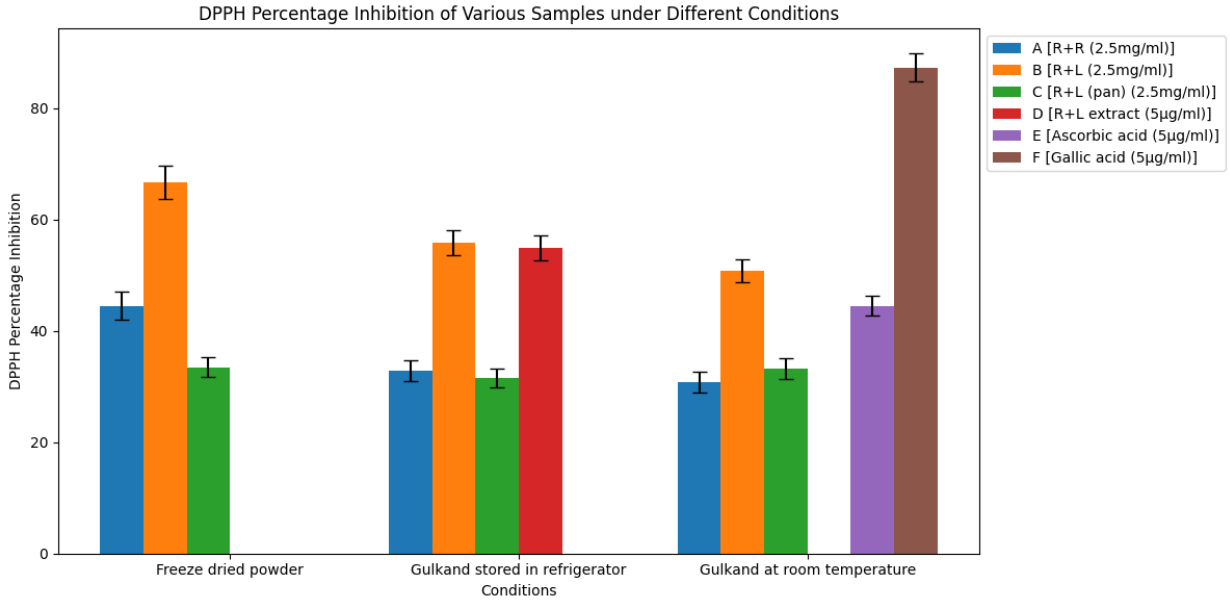

Figure 1. DPPH Percentage Inhibition of various samples under different conditions.

3.4. Total Phenolic Content

After determining the antioxidant activity of the Gulkand samples, the total phenolic compounds present in them were analyzed. Results of the same is represented in Table 3. Extract of Gulkand (R+L) contained significant amount of phenolic compounds, followed by R+L and R+R.

Table 3. Total Phenolic content of different Gulkand (n = 3) samples.

Samples	mg GAE/g extract
R+L	23.04±1.60
R+R	20.91±0.32
R+L (E)	43.25±0.54

3.5. Crude Drug - Excipient Interaction Studies

3.5.1. *Antioxidant activity of different samples with excipients*

The compatibility study results for numerous Gulkand samples and various excipients employed in the the preparation of formulations are shown in Figure 2. The antioxidant activity was reported for the first time as a factor in determining compatibility. The combinations of gulkand samples and excipients were evaluated for their antioxidant activity, and compatibility was determined based on either decrease or increase in the antioxidant activity or remained unchanged.

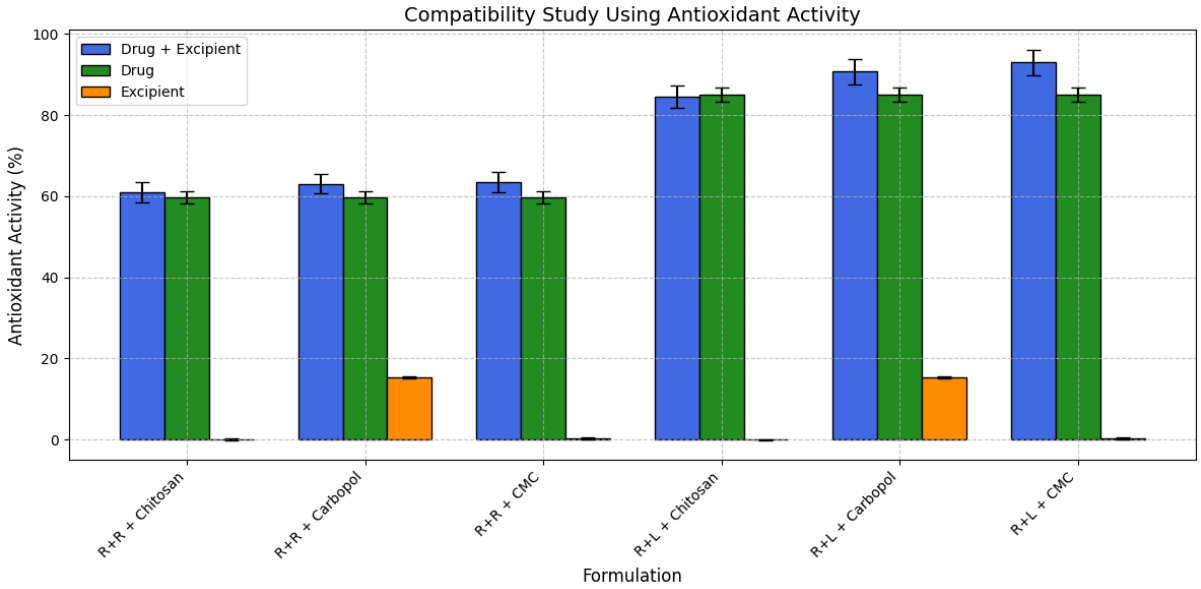

Figure 2. Compatibility study using antioxidant activity.

3.5.2. *Compatibility study of gulkand samples and excipients by FTIR*

In order to substantiate the results of the antioxidant compatibility study, FTIR analysis was performed for the same samples. The detector on the FTIR spectrometer was a deuterated triglyceride sulphate (DTGS), and IR spectra were recorded in the range 400–4000 cm^{-1} with a resolution of 4 cm^{-1}. The room was maintained at a controlled temperature of 25 degrees Celsius and relative humidity of 30%. Dry gulkand powder of sample R+L (S1) and R+L extract (S2) are weighed and compared with excipients S3 and S4. This is their graphical representation. Figure 3 depict the IR spectrum of the R+R powder sample alone and in combination with CMC. The characteristic peaks derived from the FTIR of R+R powder alone and in combination with the CMC excipient have been analyzed and reported in Table 4. This table demonstrates that the characteristic peaks of the R+R powder were unaffected by the presence of CMC excipients, indicating that the R+R powder and CMC excipients are compatible. Figure 4 depict the IR spectrum of the R+L(E) sample alone and in combination with CMC. Table 5 details the presence of characteristic FTIR peaks in R+L(E) alone and in combination with the CMC excipient. It is evident from this Table 5 that none of the characteristic peaks of the R+L(E) changed in the presence of CMC excipients, indicating that the R+L powder is compatible with CMC excipients.

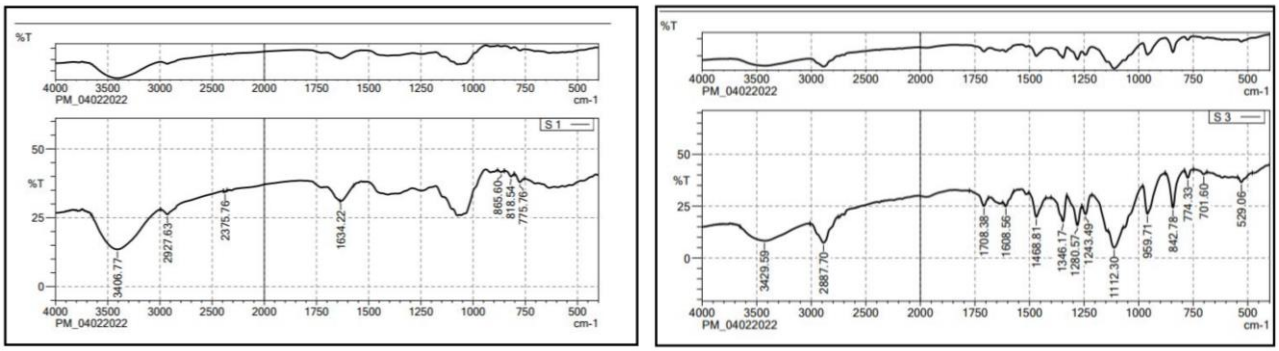

Figure 3. FTIR spectra of R+R (P) and R+R (P) with CMC.

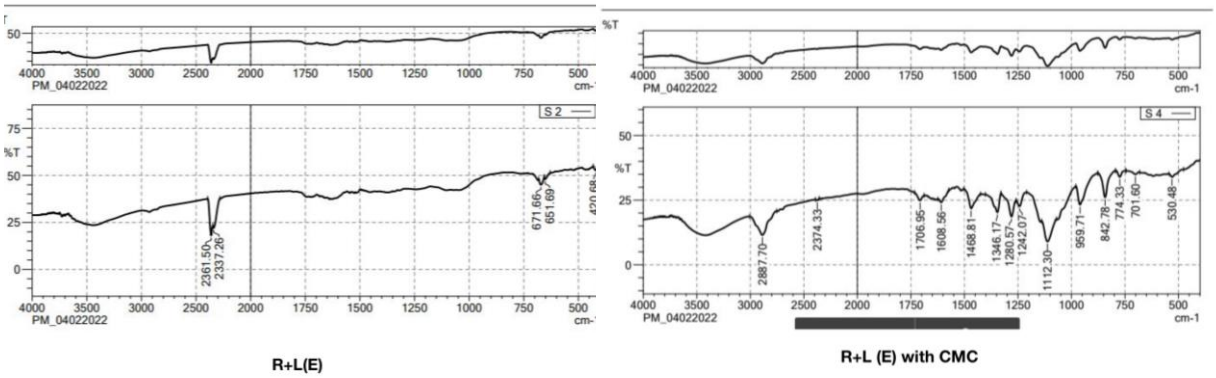

Figure 4. R+L (E) and R+L (E) with CMC.

Table 4. Comparison of R+R (P) and R+R (P) with CMC.

R+R (P)		R+R (P) and CMC	
Peaks	Functional group	Peaks	Functional group
3406.77	NH	3429.59	NH
2927.63	O-H and COOH	2887.70	O-H and COOH
2375.76	C≡N	2380.32	C≡N
1634.22	C=C, N-H, C=O	16008.56	C=C, N-H, C=O
865.6	C-H	842.78	CH,
818.54	CH, NH	842.78	CH, NH,
775.56	CH, NH, C-Cl	774.33	CH, NH, CCl

Table 5. Comparison of R+L (E) and R+L (E) with CMC.

R+L (E)		R+L (E) and CMC	
Peak	Functional group	Peak	Functional group
2361.5	C≡N	2374.33	C≡N
2337.26	C≡N	2372.26	C≡N
671.66	CH, CCL,	701.6	CH, CCl
651.69	CH, CCl, C-BR, Cl	580.6	CH, CCl, C-BR, Cl

3.7. Physical Evaluation of Gel Formulation

Buccal mucoadhesive gels were prepared utilising R+R Gulkand, R+L Gulkand, and R+L Gulkand extracts with CMC excipients and subjected to physical evaluation methods including physical appearance, centrifugation test, pH, and viscosity.

3.7.1. *Physical appearance*

The physical characteristics of the formulated gel, including its color, clarity, homogeneity, consistency, and particle presence, are detailed in Table 6. The formulations R+R (p) and R+L (p) shared identical characteristics due to the inclusion of the soluble extract in the formulations prepared in the present study. However, the gel of R + L extract was found be very clear, homogenous and with no particles present.

Table 6. Physical appearance of the different formulated gels.

Samples	Clarity	Colour	Homogeneity	Consistency	Presence of particles
R+R (p)	Not clear	Brown	Not homogeneous	Gritty	Thick particles
R+L (p)	Not clear	Dark brown	Not homogeneous	Gritty	Fine particles
R+L (e)	Not clear	Yellowish brown	Homogeneous	Clear	No particles

3.7.2. *Determination of pH and thermal tests of the prepared formulations*

The pH of all mucoadhesive gels was evaluated to ensure their suitability for the delicate buccal cavity. As indicated in the Table 7, pH of the gels remained within the neutral range specified by regulatory agencies at different tested temperature and at different duration of intervals.

Table 7. pH of the formulations at different temperature and duration.

Samples	Day 1	Day 2	Week 1	Week 2	1 month
R+L (E) 4° C	ND	6.50	6.48	6.48	6.29
R+L (E) 25° C	6.58	6.58	6.52	6.56	6.47
R+L (E) 45° C	ND	6.66	6.58	6.59	6.57
R+R (P) 4° C	ND	7.16	7.12	7.13	7.15
R+R (P) 25° C	7.11	7.10	7.10	7.09	7.10
R+R (P) 45° C	ND		7.21	7.23	7.26
R+L (P) 4° C	ND	6.81	6.80	6.81	6.82
R+L (P) 25° C	6.81	6.78	6.85	6.81	6.82
R+L (P) 45° C	ND	6.71	6.62	6.68	6.61

ND: Not Determined

3.7.3. *Centrifugal test*

Formulations were subjected to a centrifugal test to determine their stability against centrifugal force. The results indicate that none of the formulations exhibited sedimentation at different time intervals confirming their stability.

3.7.4. *Determination of viscosity*

The viscosity of the prepared gels was measured using a Brookfield DV-III viscometer. The gel prepared with rock sugar (R+R) exhibited the highest viscosity, followed by the gel prepared with liquorice and liquorice extract and the result is shown in the Table 8.

Table 8. Viscosity of the formulations.

Sample	Viscosity
R+L (P) gel	7760 cP
R+L (E) gel	2680 cP
R+R (P) gel	29720 cP

P: Powder, E: Extract and cP: Centipoise

3.8. Antioxidant Activity and Total Phenolic Content

Antioxidant activity and total phenolic content of the prepared gels were evaluated and shown in Table 9 and 10. The gel prepared with liquorice extract [(R+L (E)] exhibited higher antioxidant activity and phenolic content compared to the gels prepared with rock sugar [(R+R (P)] and liquorice [(R+L (P)].

Table 9. Antioxidant activity of different formulated gels (n=3).

Samples	% of inhibition DPPH
R+L (E) gel	86.23 ±0.93^{b}
R+L (P) gel	77.12 ±2.73^{a}
R+R (P) gel	78.09 ±0.73^{a}

Values are represented as mean ± standard deviation. Values in the same column with different alphabets in superscripts represent significant differences.

Table 10. Phenolic content determination (n=3).

Sample	GAE
R+L (E) gel	504.5 ±8.24^{c}
R+L (P) gel	375.1 ± 4.82^{a}
R+R (P) gel	391.4 ±0.47^{b}

Values are represented as mean ± standard deviation. Values in the same column with different alphabets indicates the presence of significant differences.

3.9. *In- vitro* Drug Release Study

In-vitro drug release studies was conducted using Franz diffusion cells. The release profiles of the gels are illustrated in Figure 5. The gel prepared with Liquorice extract [(R+L (E)] exhibited a sustained drug release profile compared to the other gels.

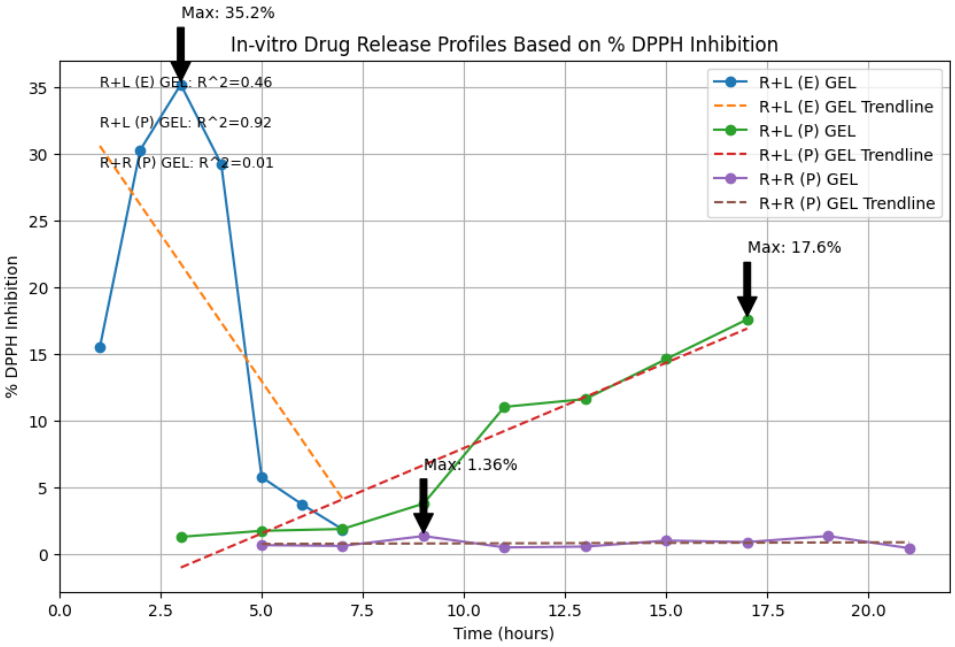

Figure 5. In-vitro drug release profile based on % DPPH Inhibition.

4. DISCUSSION

In the "Results" section, we presented an extensive evaluation of the different types of Gulkand and their characteristics, including morphological evaluation, antioxidant activity, total phenolic content, compatibility with excipients, physical evaluation of gel formulations, antioxidant activity, and phenolic content of gels, *in-vitro* drug release studies, and buccal permeation studies. The following is a discussion of the key findings and their implications: The morphological evaluation of different gulkand samples revealed intriguing differences in taste while maintaining consistent odours, texture, and the presence of broken rose petals. The variations in taste between samples can be attributed to the different sweetening agents used in their preparation, with liquorice-based gulkand exhibiting a sweet and mildly sour flavour. Despite these taste differences, the semisolid consistency and pungent odour remained consistent across all batches of Gulkand. This suggests that the base ingredients of rose petals and sugar, in different forms, provide the core characteristics of Gulkand.

4.1. Antioxidant Activity and Total Phenolic Content of Gulkand Samples

The assessment of antioxidant activity and total phenolic content provided valuable insights. Gulkand prepared with liquorice extract (R+L) demonstrated the highest antioxidant activity and phenolic content among all the samples tested. This can be attributed to the presence of liquorice, a known strong antioxidant due to the presence of various bioactive constituents (Pastorino *et al.*, 2018). Furthermore, the freeze-dried Gulkand powder exhibited superior antioxidant activity compared to the raw and frozen forms, indicating that the lyophilization process enhances the antioxidant potential compared with other drying process (Annegowda *et al.*, 2013; González *et al.*, 2023). Even the lyophilzed samples easily powdered and have smaller particle size and increased surface area that enhances the release of potent phenolic content responsible for antioxidant activity. Additionally, raw and frozen Gulkand samples are composed of higher moisture content that may be contributing for the weight of the samples compared with the freeze dried samples along with deterioration of bioactive compounds present in the Gulkand samples during the processing.

4.2. Compatibility Studies

Compatibility is crucial to ensure the stability and effectiveness of the final product. The innovative approach of using antioxidant activity to assess compatibility between Gulkand samples and excipients proved useful. This is the first study to report the application of DPPH

driven antioxidant activity to determine the compatibility of the samples and excipients used in the preparation of formulations. The results demonstrated that most combinations of gulkand samples and excipients were compatible as there was no change in the antioxidant activity of the samples after mixing with the excipients, making them suitable for the preparation of mucoadhesive gel formulations. In addition, the present method employed for the evaluation of compatibility study was found to be simple, cost effective and rapid.

4.3. Physical Evaluation of Gel Formulations

The physical evaluation of the gel formulations highlighted differences in clarity, color, homogeneity, consistency, and the presence of particles. Notably, the addition of soluble extract in the R+L (e) gel resulted in improved characteristics compared to other formulations ensuring the application of extracts rather than the crude Gulkand to be used as an ingredient in the preparation of gel. These physical properties play a role in the patient acceptability and usability of the gels for buccal administration.

4.4. pH and Stability

The pH values of the gel formulations is a crucial factor for their suitability. Results of the present study indicated that pH of the formulation remained within the neutral pH range. Additionally, the stability of the formulations against centrifugal force was also confirmed, indicating that they can withstand handling and transportation.

4.5. Viscosity

Viscosity measurements showed that the gel prepared with rock sugar (R+R) had the lowest viscosity, followed by the gel prepared with licorice extract [(R+L (E)]. Viscosity is a critical factor in mucoadhesive gels, as it influences their ability to adhere to the buccal mucosa and diffusion of the same.

4.6. Antioxidant Activity and Phenolic Content of Gel Formulations

The gels retained their antioxidant activity, and the gel prepared with licorice extract [(R+L (E)] exhibited the highest antioxidant activity among the gel formulations. This suggests that the gel formulation process did not compromise the gulkand's antioxidant properties. The determination of phenolic content further confirmed the presence of bioactive compounds in the gel formulations that may be responsible for the found antioxidant activities too.

4.7. *Ex-vivo* Drug Release Study

The *ex-vivo* drug release study provided crucial insights into how these gel formulations perform when applied to buccal mucosal membranes. The results indicated that the gel formulated with liquorice extract [(R+L (E)] achieved rapid drug release within one minute and maintained this release profile for up to five minutes. This rapid release can be attributed to the small particle size and high diffusion potential of the formulation. It suggests that R+L (E) may be well-suited for providing quick relief from oral ulcers. In accordance with our results, Jiang *et al.* (2021) reported that increased viscosity decreased the diffusion. On the other hand, the gel prepared with rock sugar [(R+R (P)] exhibited slower drug release, which may be attributed to its higher viscosity and larger particle size. This slow release could potentially lead to a longer-lasting therapeutic effect, making it a suitable option for individuals seeking sustained relief.

4.8. Antioxidant Activity and Phenolic Content of The Formulated Gels

After the gel formulations were prepared, their antioxidant activity and phenolic content were assessed. These evaluations are critical because they provide information about the formulations 'ability to retain the beneficial properties of Gulkand and its formulations. The results demonstrated that all three gel formulations retained excellent antioxidant activity. This is a promising finding, as it suggests that the gel preparation process did not compromise the formulations 'ability to scavenge free radicals and provide potential health benefits.

Furthermore, the determination of phenolic content showed that the gel prepared with gulkand [R+L (E) gel] contained the highest concentration of phenolic compounds. This aligns with the superior antioxidant activity observed in this formulation. The high phenolic content indicates that the Gulkand was effectively integrated into the gel and maintained its bioactive constituents.

4.9. Overall Implications

The comprehensive assessment of Gulkand samples carried out in the present study revealed their incorporation into mucoadhesive gel formulations as several important implications. First, it highlights the potential of gulkand, especially when prepared with liquorice extract, as a valuable component for herbal oral ulcer therapy. The different release profiles of the gel formulations (rapid vs. sustained) provide flexibility in tailoring treatment options to individual patient needs.

Second, the compatibility studies conducted using antioxidant activity as a criterion offer a novel approach to ensuring the stability of gulkand-based formulations. This approach can be applied to future research involving herbal formulations to assess their suitability for specific applications as it is found to be very simple, fast and cost effective as well as reliable.

Third, the retention of antioxidant activity and phenolic content in the gel formulations suggests that gulkand-based gels can provide not only relief from oral ulcers but also potential antioxidant and health-promoting effects.

In conclusion, the findings from this study demonstrate the potential of gulkand-based mucoadhesive gels as effective and versatile options for oral ulcer treatment. The ability to modulate drug release profiles and maintain antioxidant activity and phenolic content makes these formulations promising candidates for further research and development in the field of herbal medicine.

5. CONCLUSION

In the present investigation, three oral mucoadhesive gel formulations containing gulkand powder and extract were created. At each stage of gel preparation, samples were analysed for the presence of phenolic compounds responsible for antioxidant activity as well as the formulations' antioxidant activity. In addition, physical, chemical, and pharmacological evaluations were performed on the prepared formulations to determine their commercial viability. All of the prepared mucoadhesive gel preparations met the physical requirements. The extract of gulkand produced a substance with a high phenolic content and antioxidant activity. Within a minute, even it is released quicker from the mucosal membrane. Therefore, it will be the optimal formulation if rapid drug release is required. Even after 21 minutes, the R+R gel phytoconstituents do not discharge. This may be due to the larger particulate size of the gulkand powder. Even the R+L gel demonstrated the release of antioxidants at 11 minutes, with the process concluding at 17 minutes. Thus, both formulations assure their suitability as mucoadhesive gels for the release of antioxidant compounds necessary for the treatment of various types of mouth wounds/ulcers. Because liquorice is a potent anti-ulcer agent, the outcome of the present study was a correlation between antioxidant and anti-ulcer activity. The herbal constituents and excipients were found to be compatible through a compatibility study. To the best of our knowledge, this is the first study to report compatibility testing using antioxidant and phenolic content analysis. Further research is required to examine the in vivo anti-ulcer activity of these formulations in animal models and to determine their stability for commercialisation.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Manoj Madanahalli Ramesh: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing - original draft. **Annegowda Hardur Venkatappa:** Methodology, Supervision, and Validation.

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