

Formulation and Evaluation of Betel-Leaf-and-Clove-Based Herbal Gel

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Abstract

*This study developed and assessed an herbal gel comprising extracts of betel leaf (*Piper betle*) and clove (*Syzygium aromaticum*) for topical use. Herbal extracts were prepared by Soxhlet extraction and analyzed for yield, phytochemical profile and total phenolic content (TPC). The phytochemicals present were alkaloids, flavonoids, tannins and phenols, and glycosides, with a greater phenolic content for clove extract. Herbal gels were prepared using Carbopol 934 and assessed for properties, drug content, drug release, and biological activity. The formulations had an acceptable range of pH (6.2–6.7), uniformity, and satisfactory values of viscosity and spreadability. The drug content uniformity was between 96.2% to 98.3% and showed the homogeneity of active constituents. The in vitro release of the drug from the formulations was found to be 81.6% (F3) after 6 hours. The antimicrobial test results showed a dose-dependent increase. Formulation F3 performed best regarding drug release and physicochemical characteristics. The research suggests the formulated betel leaf and clove herbal gel is an effective, cost-effective and stable natural product for topical use.*

Keywords: Herbal gel, *Piper betle*, *Syzygium aromaticum*, phytochemical screening, Carbopol 934, in vitro drug release, drug content uniformity

INTRODUCTION

Topical drug delivery has emerged as a promising approach in contemporary pharmaceutical development because of its potential to target the drug directly at the desired site, thus improving the drug's efficacy and reducing adverse effects [1]. Gels are widely used as a topical drug delivery system due to their non-greasy texture, ease of application, increased patient acceptability and controlled release of drugs. Recently, there has been a resurgence in the development of herbal-based topical drug products due to the growing need for safe, economical and environmentally friendly alternatives to conventional synthetic drugs. Herbal plants have been known as a rich source of active ingredients with various therapeutic attributes [2]. Herbal formulations are preferred because of fewer side effects, increased acceptance by the patients and synergistic pharmaceutical effect of the phytoconstituents. In

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particular, the inclusion of plant extracts in gel formulations presents a potential avenue for developing potent formulations for topical delivery, including for dermatological and anti-infective purposes. One of the medicinal plants known for such applications is *Piper betle* (betel leaf), which is widely used in traditional medicine [3]. It contains phenolic compounds, like chavicol, chavibetol, and eugenol, that are responsible for its strong antimicrobial, antioxidant and anti-inflammatory properties. In fact, extracts of betel leaf have been found to possess promising antimicrobial activity against many pathogenic microorganisms and can, therefore, be used in topical antimicrobial preparations. Likewise, *Syzygium aromaticum* (clove) is a medicinal plant that has been traditionally used for its various health

benefits. Eugenol, the primary active component in clove, has potent antibacterial, antifungal, antiseptic and analgesic (pain-relieving) properties [4]. Clove is a well-known natural remedy for dental issues, skin infections and wound healing because of its antimicrobial and anti-inflammatory properties. The formulation containing both clove and betel leaf extracts is likely to exhibit a synergistic antibacterial effect, potentially increasing the effectiveness [5]. While there are many synthetic antimicrobial drugs available, their continued use has sometimes resulted in irritation, allergic reactions and the development of drug-resistant microorganisms. Hence, there has been a shift towards safe, environmentally friendly herbal agents. In particular, herbal gels offer a suitable vehicle for application of herbal actives, as this guarantees stability, application convenience and better skin penetration [6]. Thus, the aim of the current study is to prepare and assess the herbal gel formulation of betel leaf and clove extracts (Figure 1). The aim of this research is to formulate a stable and active gel preparation and assess the physical and chemical characteristics and antimicrobial activity of the preparation. Such research is likely to lead to the development of a natural, safe, and efficient topical antimicrobial agent having a wide range of pharmaceutical and cosmetic uses.

MATERIALS AND METHODS

Materials

Fresh leaves of *Piper betle* and dried flower buds of *Syzygium aromaticum* were obtained from a well-known authenticated herbal dealer in Ahmedabad, Gujarat, India. The botanical authenticity of the plants was checked by a taxonomist, and voucher specimens were kept for reference. Carbopol 934 was used to make the gel, triethanolamine facilitated the neutralization, and glycerin and methyl paraben were used as a humectant and preservative, respectively. Analytical grade ethanol was used as an extraction solvent. Folin–Ciocalteu reagent, gallic acid standard and other analytical chemicals were obtained from usual sources. Analytical grade chemicals were used.



Figure 1. Raw plant materials used in formulation (Betel leaf and Clove).

Preparation and Standardization of Plant Extracts

Preparation of Extracts

Fresh leaves of *Piper betle* and dried buds of the flower of *Syzygium aromaticum* were washed with distilled water to remove dirt and dried in a well-ventilated place at room temperature ($25 \pm 2^\circ\text{C}$) for 5–7 days or until constant weight was obtained. The powdered plant material was further coarsely crushed by a mechanical grinder and sieved to get uniform sized samples. Known weight (100 g) of plant powders were taken in round bottom flasks and Soxhlet extracted with 95% v/v ethanol as solvent for 6–8 hours. The process of extraction continued until the solvent in the siphon tube became colorless. The extracts were then filtered through Whatman No. 1 filter paper and evaporated to dryness in a rotary vacuum evaporator at $40\text{--}45^\circ\text{C}$ to avoid the thermal degradation of the active constituents. The extracts were then evaporated to dryness to form semi-solid masses which were stored in airtight containers in a desiccator for future studies [7].

Determination of Percentage Yield

The yield of the extracts was calculated to determine the yield of the extraction process. The yield was calculated as the ratio of the dried extract obtained to the weight of crude drug as described by the equation:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of crude drug (g)}} \times 100$$

Organoleptic Evaluation

Organoleptic qualities, such as color, odor, and texture, were determined. These were assessed visually and organoleptically in controlled conditions to ensure consistency and no signs of deterioration.

Preliminary Phytochemical Screening

We performed the preliminary phytochemical analysis of ethanolic extracts from *Piper betle* and *Syzygium aromaticum* for different classes of secondary metabolites through standard procedures. Tests were conducted for identification of alkaloids (Dragendorff's and Mayer's tests), flavonoids (Shinoda test), tannins (Ferric chloride test), phenolic compounds, glycosides and saponins. The colour reactions were observed, and their respective intensities were recorded to give a qualitative assessment of the phytoconstituents, which will be useful for pharmacological studies [8].

Total Phenolic Content (TPC)

The total phenolic content (TPC) in the extracts was measured by the Folin–Ciocalteu colorimetric method, as modified. In summary, 1 mL of the extract solution (suitably diluted) was combined with 5 mL of Folin–Ciocalteu reagent (diluted 1:10 with distilled water). Then, 4 mL of 7.5% (w/v) sodium carbonate solution was added, and the solution was allowed to stand at room temperature and protect from light for 30 minutes. The blue complex was measured at 765 nm absorption wavelength using a UV-Visible spectrophotometer, relative to an appropriate blank. Gallic acid was used to prepare the standard curve, and the amount of phenolic content was expressed as milligrams of gallic acid equivalent per gram of sample (mg GAE/g extract). All measurements were carried out in triplicate [9].

Formulation of Herbal Gel

The formulations of herbal gel (F1 to F4) comprised of extracts of *Piper betle* and *Syzygium aromaticum* are shown in Table 1. The goal of the formulations was to assess the influence of different extract concentrations on the physicochemical properties and effectiveness of the formulations.

Table 1. Composition of betel leaf and clove-based herbal gel formulations (F1–F4).

S. N.	Ingredients	F1 (% w/w)	F2 (% w/w)	F3 (% w/w)	F4 (% w/w)
1	Carbopol 934	1.0	1.0	1.0	1.0
2	Betel leaf extract	1.0	2.0	3.0	4.0
3	Clove extract	0.5	1.0	1.5	2.0
4	Glycerin	5.0	5.0	5.0	5.0
5	Methyl paraben	0.2	0.2	0.2	0.2
6	Triethanolamine	q.s.	q.s.	q.s.	q.s.
7	Distilled water	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100

Method of Preparation

Herbal gel formulations were developed using Carbopol 934 as a major gelling agent using a basic formulation procedure. As outlined in Table 1, Carbopol 934 (1% w/w) was weighed and gradually added to distilled water with constant stirring (to avoid lump formation). The polymer dispersion was left to soak for 24 hours at room temperature ($25 \pm 2^\circ\text{C}$) for the polymer to swell. Then, glycerin (5% w/w) and methyl paraben (0.2% w/w) were added to the polymer dispersion with continuous mixing to get a homogenous blend. The specific amount of betel leaf extract and clove extract (as per formulations

F1–F4 in Table 1) was slowly added and thoroughly mixed to obtain uniformity. Triethanolamine was gradually added while the mixture was stirred, neutralising the Carbopol dispersion to form a gel. The formulation's pH was carefully measured and then adjusted to 5.5–7.0 for optimal compatibility with skin pH. The gels were left to stand for a period to allow removal of any air bubbles. The formulations were then transferred to air-tight and light-resistant containers and stored at room temperature for further analysis [10].

Evaluation of Herbal Gel

Physical Appearance

The prepared gel formulations were assessed for organoleptic properties such as color, transparency, uniformity, and consistency visually under ambient light. They were also checked for any sediment, cloudiness or grittiness for homogeneity [11].

pH Measurement

The pH of the formulations was assessed using a digital pH meter. A known weight (about 1 g) of the gel was accurately weighed and dispersed in 10 mL distilled water to give a uniform suspension. The pH was measured at room temperature ($25 \pm 2^\circ\text{C}$) by immersing the electrode in the sample. Triplicates were carried out for accuracy [12].

Viscosity

The viscosity of the gels was determined using a Brookfield viscometer with a suitable spindle. This was done at a fixed temperature ($25 \pm 1^\circ\text{C}$) and spindle speed. Data was noted when the reading was steady and the average viscosity was determined by taking readings for three times [13].

Spreadability

The spreadability of the formulations was measured using the glass slide method. A fixed amount of the gel was placed between two glass slides, and a certain weight was placed on the upper slide [14]. The time taken for the upper slide to move a certain distance was measured. Spreadability was calculated using the following equation:

$$S = \frac{M \times L}{T}$$

where: S = Spreadability (g·cm/sec), M = Weight tied to upper slide (g), L = Length moved by the slide (cm) and T = Time taken (sec).

Extrudability

Extrudability was assessed by filling the formulations into collapsible (aluminum) tubes. The weight was maintained and extrusion volume measured in a fixed time period. Qualitative measures of extrudability (excellent, good or poor) were based on the ease of extrusion.

Drug Content Uniformity

Assay of drug content uniformity was done to confirm uniform distribution of the phytoconstituents in the gel. A weighed amount (about 1 g) of gel was dissolved in proper solvent (ethanol or phosphate buffer) with sonication (if needed) and filtered. The sample was suitably diluted and absorbance was measured by UV-Visible spectrophotometer at a suitable wavelength. The drug content was determined from a suitable calibration curve, and the percentage drug content present was calculated [15].

In Vitro Drug Release Study

The in vitro release experiments were performed using a Franz diffusion cell and a dialysis membrane (which has been soaked in the diffusion medium for 12 hours). The receiver compartment of the diffusion cell was filled with phosphate buffer (pH 6.8) and kept at a temperature of $37 \pm 0.5^\circ\text{C}$, being continuously stirred (magnetic stirrer). An appropriate amount of gel was added to the donor chamber.

At specified time points, samples were removed from the receptor compartment and replaced with fresh buffer solution (sink conditions). The amount of drug released was determined spectrophotometrically at an appropriate wavelength and cumulative drug release was determined. Experiments were performed in triplicate [16].

RESULTS AND DISCUSSION

Evaluation of Plant Extracts

Percentage Yield and Organoleptic Properties

Macro-extraction of plant materials resulted in the formation of semi-solid materials with distinctive odors and colours, confirming the successful extraction of phytoconstituents. The obtained yields and organoleptic characteristics of the extracts are shown in Table 2. The findings suggest that the clove extract has a higher percentage yield ($14.3 \pm 0.5\%$ w/w) compared to the extracts of betel leaf ($11.8 \pm 0.6\%$ w/w), which could be due to the higher content of ethanol-soluble substances, including essential oils and polyphenols in the clove buds. These organoleptic properties further confirm the successful nature of the extraction method; the dark green colour of the betel leaf extract implies the presence of chlorophyll and polyphenolic substances, while the brownish colour and intense spice-like aroma of the clove extract are suggestive of the eugenol-containing volatile compounds. Moreover, the semi-solid mass of the two extracts indicates that the solvent evaporation process was successful under controlled conditions, thereby avoiding undesirable degradation of thermolabile phytoconstituents. The absence of any unusual smell and/or colour of the extracts also confirms their stability and integrity, and thereby suitability for further processing and analysis.

Table 2. Percentage yield and organoleptic properties of extracts.

Extract	% Yield (w/w)	Color	Odor	Consistency
Betel leaf extract (<i>Piper betle</i>)	11.8 ± 0.6	Dark green	Aromatic	Semi-solid.
Clove extract (<i>Syzygium aromaticum</i>)	14.3 ± 0.5	Brownish	Strong spicy	Semi-solid.

Phytochemical Screening

The preliminary phytochemical screening revealed the presence of key bioactive compounds in the herb extracts as shown in Table 3. Alkaloids, flavonoids, tannins, phenols, and glycosides were detected in both the betel extracts and clove extracts, with the absence of saponins. This analysis is in agreement with the phytochemical constituents of *Piper betle* and *Syzygium aromaticum*. Interestingly, the clove extract showed a relatively higher content of phenolic compounds (+++) possibly due to the presence of eugenol. The identification of phenolic compounds, flavonoids and tannins is particularly important, as these components are well known for their antimicrobial properties by mechanisms such as microbial cell membrane disruption, denaturation of microbial proteins, and inactivation of microbial enzymes. Thus, the phytochemical profile suggests that both extracts are likely to possess antimicrobial activity, validating the use of these extracts in the formulation of the herbal gel.

Table 3. Phytochemical screening of extracts.

Phytoconstituent	Betel extract	Clove extract
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Phenols	+	+++
Glycosides	+	+
Saponins	–	–

Note: + = present, +++ = strongly present, – = absent.

Total Phenolic Content (TPC)

Table 4 shows the total phenolic content of the extracts, and the clove extract (146.7 ± 3.5 mg GAE/g) had a greater phenolic content than the betel extract (82.4 ± 2.1 mg GAE/g).

Table 4. Total phenolic content of extracts.

Extract	TPC (mg GAE/g extract)
Betel extract	82.4 ± 2.1
Clove extract	146.7 ± 3.5

Evaluation of Herbal Gel Formulations

Physical Evaluation

Each formulation (F1–F4) was smooth, uniform and lump-free. Suspensions' color varied from green (F1) to dark greenish-brown (F4) because of increasing pomegranate extract concentration (Figure 2).

**Figure 2.** Betel leaf and clove based herbal gel.

pH, Viscosity, and Spreadability

All formulations (F1–F4) were uniform, lump-free, free-flowing and exhibited darker color with increasing extract concentrations. Table 5 shows pH (6.2–6.7) was suitable for skin, and viscosity increased from F1 to F4 because of increased extract concentration and interactions with the Carbopol polymer. Spreadability slightly decreased with increased viscosity, but all formulations were still easily spreadable.

Table 5. Physicochemical evaluation of gel formulations.

Formulation	pH (± SD)	Viscosity (cP)	Spreadability (g·cm/sec)
F1	6.2 ± 0.1	4320 ± 85	18.6 ± 0.5
F2	6.4 ± 0.1	4510 ± 90	17.9 ± 0.6
F3	6.5 ± 0.1	4785 ± 95	17.2 ± 0.4
F4	6.7 ± 0.1	5020 ± 110	16.5 ± 0.5

Drug Content Uniformity

Drug content uniformity (from Table 6 and Figure 3) shows that all the formulations were uniformly distributed with acceptable percentage (95–105%) of drug, indicating good mixing and homogeneity of the gel network, with slightly higher drug content uniformity among others of F3 formulation.

Table 6. Drug content uniformity.

Formulation	Drug Content (%)
F1	96.2
F2	97.5
F3	98.3
F4	97.8

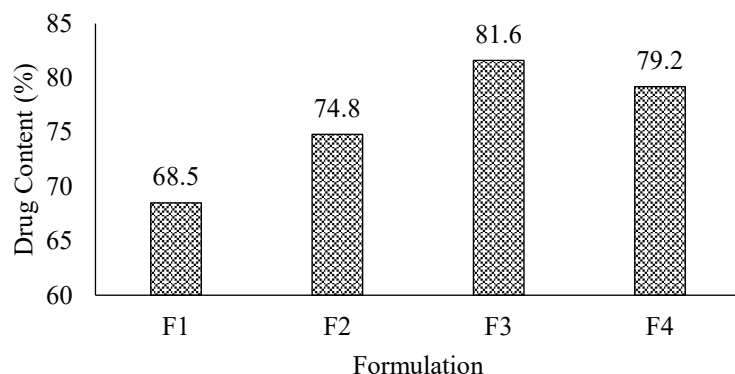


Figure 3. Drug content of herbal gel formulations (F1–F4).

In Vitro Drug Release

The cumulative drug release profile (Table 7 and Figure 4) ranges from 10 to 12% with a steady rise from F1 to F3 suggesting increased diffusion with increasing quantity of extract; however, a slight drop in F4 may be due to the increased viscosity, impeding the drug diffusion; thus, F3 showed the perfect combination of viscosity and drug release.

Table 7. Cumulative drug release (% at 6 hrs).

Formulation	% Drug release
F1	68.5
F2	74.8
F3	81.6
F4	79.2

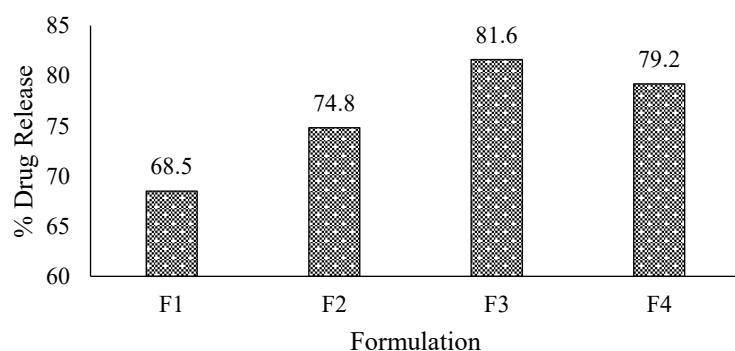


Figure 4. In vitro drug release profile of herbal gel formulations (F1–F4).

CONCLUSION

This study involved the development and characterization of a herbal gel formulation that includes extracts of betel leaf (*Piper betle*) and clove (*Syzygium aromaticum*). The formulations showed satisfactory physical properties such as pH, viscosity, spreadability and uniformity. Phytochemical screening revealed the presence of active compounds that contribute to the therapeutic effect and total phenolic content suggested a higher amount in clove extract. F3 was found to be the ideal formulation with a controlled release, drug distribution, and good application characteristics. In summary, the herbal gel holds promise as an effective natural topical formulation and could be used as a substitute for synthetic formulations.

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Conflict of Interest

NIL.

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