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FORMULATION AND EVALUATION OF TOPICAL EMULGEL CONTAINING ETHANOLIC EXTRACT OF *Piper betle Linn.* LEAF FOR WOUND HEALING

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ABSTRACT

The present study aimed to develop and evaluate a topical emulgel formulated with the ethanolic extract of *Piper betle Linn*. leaves for wound-healing purposes. The extract was obtained through Soxhlet extraction using ethanol, producing a semi-solid mass abundant in phytoconstituents. Preliminary phytochemical screening revealed the presence of carbohydrates, proteins, alkaloids, glycosides, terpenes, steroids, flavonoids, tannins, saponins, and phenolic compounds. Preformulation studies indicated favourable organoleptic characteristics, good solubility in ethanol, methanol, DMSO, and coconut oil, and a λmax of 272 nm. FTIR analysis confirmed that the functional groups remained intact, showing no chemical degradation after incorporation into the emulgel base. Coconut oil served as the oil phase, while a combination of Tween 80 and Span 80 was optimized to achieve a stable emulsion. Six emulgel formulations were prepared by varying the concentration of Carbopol 940, among which formulation F4 was selected as the optimized batch based on physicochemical evaluations. F4 displayed a pale-yellow creamy texture, excellent homogeneity, absence of phase separation, uniform globule distribution, high drug content (98.2%), a skin-friendly pH (5.70), good spreadability (8.67 g·cm/sec), and appropriate viscosity (14,150 cP). *In vitro* release studies demonstrated a sustained release pattern, with 92.3% of the drug released over 8 hours. The wound-healing potential was assessed using the excision wound model in Wistar rats. The optimized emulgel exhibited a significant improvement in wound contraction (63% by Day 9) and achieved complete closure by Day 15. In comparison, the crude *Piper betle Linn*. extract showed 98.5% closure by Day 18, while the standard drug (povidone-iodine 5% w/w) reached 100% closure by Day 18. Overall, the results indicate that incorporating *Piper betle Linn.* extract into an emulgel base significantly enhances its wound-healing performance, confirming its potential as a stable, safe, and effective herbal formulation for topical wound care.

KEYWORDS: *Piper betle Linn.*, ethanolic extract, emulgel, Carbopol 940, excision wound model, wound healing.

INTRODUCTION

Herbal remedies have been employed worldwide for centuries in the management of various infectious diseases. Utilizing medicinal plants as novel antibacterial agents offers several benefits, such as improved safety, wider accessibility, and a lower likelihood of adverse effects or dependency.[1] A well-known example is the betel leaf, which is referred to by different names in various regions of India and other countries, based on its features like structure, color, aroma, taste, and size. These names include Venmony, Magadhi, Salem, Kauri, Banarasi, Mysore, Bagerhati, Bangla, Kasi, Desavari, Meetha, Ghanagete, Sanchi, and Kapoori.[2] More than 700 species of *Piper betle Linn*. are found across the world, with about 30 species reported in India. The plant is widely cultivated in India and also in countries such as

Sri Lanka, Malaysia, Indonesia, the Philippines, and regions of East Africa.[3] The plant grows well in shaded areas and is a perennial climber with leaves measuring 2–4 inches in width and 4–7 inches in length. It produces both male and female flowers. The spadix appears singly at the tip, positioned opposite the leaves, with a length of 5–15 cm and a width of 2–5 cm. The male spike is about 1.5–3 cm long and contains two short stamens, while the female spike measures 2.5–6 cm in length and carries three to five pistils that are white to yellowish-green in color.[4] *Piper betle Linn*. is regarded as a safe natural antioxidant. Chewing or consuming its leaves activates the salivary glands, enhancing salivation, which marks the first step in digestion. Extracts and isolated compounds of *Piper betle Linn.* possess multiple therapeutic properties, including antiseptic, antibacterial, antioxidant, anti-inflammatory, anticancer, and immunomodulatory activities. The plant is rich in phytochemicals such as alkaloids, flavonoids, steroids, saponins, tannins, sugars, diastases, and essential oils. Furthermore, Ayurvedic texts indicate that betel leaves and their constituents may help regulate heart rate by relaxing blood vessels, thereby contributing to the reduction of hypertension.[5]

Emulgel are emulsions, either oil phase dispersed in water/aqueous as continuous phase or water/aqueous phase dispersed in oil as continuous phase, which is converted to gel by mixing with a suitable polymer. Emulgel is a most promising vehicle for the delivery of hydrophobic drugs. The Emulgel in other words is a combination of emulsion and gel. Emulgel provides significant advantages over both new and conventional vesicular systems in a variety of ways: Having a long shelf life, being emollient, non-staining, water-soluble, thixotropic, greaseless, easily spreadable, rapidly removable, translucent, and visually appealing.

Rationale of Emulgel as a Topical Drug Delivery System

Numbers of medicated products are applied to the skin or mucous membrane that either enhance or restore a fundamental function of skin or pharmacologically alter an action in the underlined tissues. Such products are referred as topical or dermatological products. Many widely used topical agents like ointments, creams lotions have many disadvantages. They have very sticky causing uneasiness to the patient when applied. Moreover, they also have lesser spreading coefficient and need to apply with rubbing. And they exhibit the problem of stability also. Due to all these factors within the major group of semisolid preparations, the use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations. A gel is colloid that is typically 99% wt. liquid, which is immobilized by surface tension between it and a macromolecular network of fibers built from a small amount of a gelating substance present. In spite of many advantages of gels a major limitation is in the delivery of hydrophobic drugs. So, to overcome this limitation an emulsion-based approach is being used so that even a hydrophobic therapeutic moiety can be successfully incorporated and delivered through gels.[6,7]

A wound can be described as the disruption or loss of cellular, anatomical, or functional continuity in living tissues. Wound healing is a natural biological process triggered by injury and usually completed with the formation of a scar. Therefore, healing serves as a vital survival mechanism aimed at restoring normal anatomical structure and function.[8]

Under normal conditions, wound healing occurs through a series of processes that primarily involve a connective tissue response. The process begins with an acute inflammatory phase, followed by the production of collagen and extracellular macromolecules, which are later remodelled to form a scar.[9] The process of wound healing occurs in different phases such as coagulation, epithelisation, granulation, collagen formation and tissue remodelling.

MATERIALS AND METHODS

a) Materials

The Coconut oil, span 80, Propyl paraben was purchased from Karnataka fine chem, Bangalore. Carbopol 940, Propylene glycol was purchased from Sisco Research Laboratories. Tween 80 were purchased from S d FINE CHEM. Methyl paraben were purchased from Merk Specialities Private Limited.

Plant sample collection and identification

Piper betle Linn. leaves were collected from locally from Mysuru, Karnataka, India. *Piper betle Linn*. leaves were sent to Department of Botany, Sarada Vilas College Krishnamurtypuram, Mysuru, Karnataka, India. for species authentication.

Preparation of *Piper betle Linn.* Leaf Extract

Fresh *Piper betle Linn*. leaves were thoroughly washed under running water to remove any surface impurities. The cleaned leaves were then cut into small pieces and dried under shade for 3–4 weeks to preserve their phytoconstituents. Once completely dried, the leaves were ground into a fine powder using an electric blender. A total of 15 g of the powdered leaves was subjected to Soxhlet extraction using 300 ml of ethanol as the solvent. The extraction process was carried out at a controlled temperature of 65°C for 3 hours, completing approximately 10 cycles. The obtained extract, which appeared dark green in color, was then concentrated by drying in a hot air oven at 40°C for three days until all the solvent had evaporated, yielding a dry crude extract for further formulation.[10,11]

Phytochemical screening of extract

Preliminary phytochemical tests were performed on the crude extracts to identify constituents such as carbohydrates, proteins, alkaloids, glycosides, terpenes, steroids, flavonoids, tannins, and saponins using standard methods.

b) METHODOLOGY

1. Preformulation studies

1.1 Organoleptic evaluation

Organoleptic evaluation of the drug extract was conducted to assess its sensory attributes, including colour, odour, taste and texture.

1.2 Solubility studies

Solubility studies of the drug were carried out in different types of solvents which are used for further study. Initially 500 mg of *Piper betle Linn*. extract was dissolved in 10 ml of suitable solvents like ethanol, methanol, isopropyl myristate, DMSO, glycerol and water stirred using magnetic stirrer for a period of 10 minutes in order to ensure the solubilisation of drug in a particular solvent.

1.3 Determination of Maximum wavelength (λ max)

UV spectrophotometer (Shimadzu, UV-1900i Japan, UV-spectrophotometer) was used to determine λ max of ethanolic extract of *Piper betle Linn*. in scan mode with the scanning range of 200-400 nm.

1.4 Preparation of standard calibration curve

The standard solutions with concentrations of 2, 4, 6, 8 and 10 ml µg/ml were analysed using UV spectrophotometer (Shimadzu, UV-1900i Japan). against a blank (Ethanol) measuring absorbance at 272 nm. A calibration curve was created by plotting absorbance against the concentration of the standard solutions, and the regression equation was derived. This experiment was conducted in triplicate to ensure accuracy.[12]

1.5 Compatibility study by FT-IR Spectrophotometer

The IR spectrum of pure *Piper betle Linn.* extract was obtained to assess its compatibility with excipients used in the formulation. A physical mixture of the drug and various polymers was prepared for this purpose. Approximately 2-3 mg of the sample was mixed with potassium bromide, dried at 40-50°C, and compressed into a transparent pellet under 10 tonnes of pressure using a hydraulic press. The prepared pellet was analysed in an FTIR spectrophotometer, scanning within the 4000-400 cm^{-1} range.[13]

2. FORMULATION DEVELOPMENT

2.1 Preparation of Emulsion and Stability

The emulsion was formulated for incorporation into the gel base using the Hydrophile-Lipophile Balance (HLB) system to determine the optimal ratio of components. Emulsifiers contain both hydrophilic and lipophilic segments, and their balance defines the HLB value. This value can be either calculated or experimentally determined.

Selection of Emulsifiers Suitable to RHLB of Oil Phase

According to the HLB System, all fats and oils have a Required HLB. RHLB of the oil phase, Coconut oil – 8 An emulsifier blend is prepared such that it will have a HLB of 11.50. For better stability of emulsion, blends of high HLB and low HLB emulsifiers used for the formulation of emulsion by using Span 80 (RHLB=4.3) and Tween 80 (RHLB=15). To determine the percentage composition of two emulsifying agents, A combination of high and low HLB emulsifier is usually used to get desired HLB (denoted by X). It can be calculated as,

$$\%\ A = \frac{(X - HLB_B)}{HLB_A - HLB_B} \times 100$$

$$\%\ B = 100 - \%\ A$$

HLB_A = RHLB of Tween 80 HLB_B = RHLB of Span 80 X = Desired HLB value.

The HLB System says that a blend of Tween 80 = 34.57% and Span 80 = 65.42% would be needed to emulsify an oil phase that has a required HLB of 8.

Preparation of emulsion with RHLB emulsifier blend ratio

From the above obtained emulsifier blend ratio, the emulsion was prepared with the Smix concentration of 0.5% - 5% which was further checked for its stability and one single concentration was chosen for the further formulated batches.

Ternary phase diagram

The goal of this study is to provide an overview on the importance and assessment of ternary plot, a vital tool for selecting suitable formulations in the development of stable emulsions. Such emulsions are designed to improve the stability of bioactive compounds or hydrophobic drugs. Constructing a ternary phase diagram is crucial for identifying and optimizing the component ratios required for effective emulsion formulation.

Ternary plot

The aqueous titration method was employed to construct the ternary plot consisting of oil, surfactant/co-surfactant mixture (Smix), and water. Double-distilled water was used as the aqueous phase for emulsion preparation. Ten different combinations of oil and Smix (as shown in Table 1) were mixed and vortexed for 5 minutes to obtain a transparent, homogeneous mixture. Distilled water was then added dropwise while monitoring changes in transparency and flowability. The point at which the mixture turned from transparent to turbid was recorded. The phase diagrams showing the largest emulsion regions were selected for emulsion formulation, and ternary plots were generated using TernaryPlot.com.[14,15,16]

Table no 1: Different combinations of oil and Smix.

Sl.no	Oil (%)	Smix (%)
1	19.5	0.5
2	19	1
3	18.5	1.5
4	18	2
5	17.5	2.5
6	17	3
7	16.5	3.5
8	16	4
9	15.5	4.5
10	15	5

2.2 Emulgel formulation

To prepare the emulgel of *Piper betle Linn*. leaf extract, the oil phase was prepared by gently heating 15 mL of coconut oil, and 1 g of *Piper betle Linn.* leaf extract was dissolved into it. Subsequently, 5 mL of Smix (a pre-blended mixture of Tween 80 and Span 80) was added to the oil phase with continuous stirring until a clear, uniform solution was obtained.

In a separate beaker, the aqueous phase was prepared by dissolving 0.03 g of propyl paraben and 0.01 g of methyl paraben in a small quantity of warm distilled water, followed by the addition of 10 mL of propylene glycol as a permeation enhancer, and the mixture was stirred thoroughly. Separately, 2% w/w Carbopol 940 was dispersed in sufficient distilled water with continuous stirring to prevent clumping and allowed to fully hydrate for 2–3 hours (or overnight).

Once the oil and aqueous phases were prepared, the oil phase was slowly added into the aqueous phase with continuous stirring using a high-speed homogenizer to form a stable oil-in-water emulsion. Finally, this emulsion was gradually incorporated into the Carbopol gel base with gentle stirring. The pH of the formulation was adjusted to around 6–7 using a suitable neutralizing agent such as triethanolamine to activate gel formation, and stirring was continued until a smooth, consistent emulgel was obtained.

The composition of the various formulations is detailed in Table 2.

Table no 2: Composition of Emulgel (F1 – F6).

Ingredient (%w/w)	**Formulation code**					
	F1	**F2**	**F3**	**F4**	**F5**	**F6**
	Emulsion					
Piper betle Linn. extract	01 gm	01 gm	01 gm	01 gm	01 gm	01 gm
Coconut oil	15 ml	15 ml	15 ml	15 ml	15 ml	15 ml
Tween 80	3.52 ml	3.52 ml	3.52 ml	3.52 ml	3.52 ml	3.52 ml
Span 80	6.48 ml	6.48 ml	6.48 ml	6.48 ml	6.48 ml	6.48 ml
Distilled water	qs	qs	qs	qs	qs	qs
Propylene glycol	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml
Gel base						
Carbopol 940	0.5	1	1.5	2	2.5	3
Distilled water	qs	qs	qs	qs	qs	qs
Propyl paraben	0.01gm	0.01gm	0.01gm	0.01gm	0.01gm	0.01gm
Methyl paraben	0.03 gm	0.03 gm	0.03 gm	0.03 gm	0.03 gm	0.03 gm
Emulsion - Gel	01: 01	01: 01	01: 01	01: 01	01: 01	01: 01
Triethanolamine was added to adjust the pH						

3. Characterization of Emulgel

a) Physical evaluation

All the prepared formulations were examined visually for color, appearance, homogeneity, and phase separation.

b) Microscopic study

The emulgel was diluted, placed on a glass slide, and observed under a light microscope at 40x magnification to examine the globular structures present within the gel base.

c) Determination of Gel Strength

The strength of the formulated gel at different concentrations was evaluated using the penetration method. In this method, a metal ball of known weight and size was dropped into the gel contained in a 100 ml graduated cylinder, and the time (in seconds) taken by the ball to penetrate a distance of 1 cm was measured.[17,18]

d) Determination of drug content

The drug concentration in the emulgel was determined using a UV spectrophotometer. 1 g of the formulation was transferred to a 10 ml volumetric flask, followed by the addition of methanol or ethanol. The mixture was shaken thoroughly, and the volume was made up to 10 ml with ethanol. The resulting solution was filtered to obtain a clear filtrate. The absorbance was measured at 272 nm using a UV spectrophotometer, and the procedure was repeated three times to determine the average drug content in each formulation. The amount of active ingredient present in 1 g of formulation was calculated using the calibration curve by correlating the absorbance with the drug concentration.[19,20,21]

Drug Content = Concentration × Volume taken × Dilution factor x Conversion factor

e) pH determination

pH evaluation is a crucial parameter, particularly for topical formulations. The emulgel should have a pH between 5 and 7 to match the natural skin environment. A formulation that is too acidic or too basic may cause irritation to the skin. The pH of the emulgel was measured using a digital pH meter at room temperature, which was initially calibrated with standard pH 4 and pH 7 buffer solutions.

The pH of the formulation was determined using both the direct and dilution methods.

- **Direct Method:** 1 g of the sample were placed in a glass vial, and the pH meter electrode, previously rinsed with distilled water, was immersed in the formulation. Three readings were taken for each sample, and the average pH was calculated.
- **Dilution Method:** A 10% dispersion was prepared by dissolving 1 g of the formulation in 10 ml of purified water. The pH electrode was immersed in the dispersion, and the stabilized reading was recorded. This procedure was repeated three times to obtain the average pH value.[22]

f) Spreadability

An essential quality of a dermatological preparation is good spreadability, which indicates how easily a gel spreads over the skin or affected area. The therapeutic effectiveness of a formulation is influenced by its spreadability; therefore, evaluating this property is important.

The spreadability of *Piper betle Linn.* emulgel was assessed using the glass-slide method. A fixed amount of gel (0.5–1.0 g) was placed at the center of a clean glass slide and covered with another slide. A standard weight of 500 g was applied for 5 minutes to ensure uniform spreading and remove trapped air. The upper slide was then connected to a string with a fixed weight, and the time taken for the slide to travel a predetermined distance of 7.5 cm was recorded.[23]

g) Viscosity determination

The emulgel formulation was subjected to rheological evaluation using a manual Brookfield viscometer (Brookfield RV viscometer) with spindle No. 7 to determine viscosity in centipoises at room temperature, and the flow pattern was analysed by plotting viscosity against rotational speed (RPM). The emulgel was placed in the sample holder, and the appropriate spindle was carefully lowered into the center of the sample, ensuring it did not touch the bottom of the container. The spindle was then rotated at various speeds, and the viscometer measured the resistance to rotation to provide viscosity readings. Measurements were recorded after 5 minutes.[24]

h) Swelling index

The performance of a polymer in pharmaceutical formulations is enhanced when the macromolecule is fully swollen, improving rheological properties, suspension stability, and emulsification in topical preparations.

The swelling behavior of the emulgel was evaluated by placing 1 g of the formulation on porous aluminum foil and immersing it in a 100 ml beaker containing 20 ml of 0.1N sodium hydroxide (NaOH). At predetermined time intervals, the emulgel along with the foil was removed, surface water was gently blotted using filter paper, and the sample was weighed until a constant weight was achieved. The initial weight of the emulgel at zero time (W_0) and the weight of the swollen emulgel at each time point (W□) were recorded.[25]

The Swelling index was calculated using:

$$\text{Swelling index (SW\%)} = \left[\frac{Wt - Wo}{Wo}\right] \times 100$$

Where,

(SW)% = Equilibrium percent swelling

W_t = Weight of swollen emulgel after time,

W_o = Original weight of emulgel at zero time.

i) In vitro diffusion study

An in vitro release study was carried out for the time period of 6 hours. Dialysis membrane diffusion model was used to determine the invitro drug release profile of *Piper betle Linn* extract from the emulgel formulation. The apparatus consists of a glass cylinder with both the ends open, 10 cm in height, was used as a permeation cell. A cellophane membrane soaked in distilled water (24 hours before use) was fixed to the one end of the cylinder with aid of an adhesive. An accurately weighed amount of emulgel (equivalent to 125 mcg) was placed in the cell (donor compartment) and the cell was immersed in a receptor compartment containing 50 ml of phosphate buffer (pH 7.2). The whole assembly was fixed in such a way that the lower end of the cell containing emulgel was just touched (1-2 mm deep) to the diffusion medium. The diffusion medium was continuously stirred with a magnetic stirrer at 50 rpm and then up to 6h (0.5, 1, 2, 3, 4, 5 and 6 hours) at different time interval the sample was withdrawn from receptor medium and maintained the sink condition. Fraction of diffusion medium each of 2ml was removed at prescribed time points. The removed diffusion medium solution was restored by same amount of diffusion medium. The sample was analyzed by using Shimadzu UV visible spectrophotometer at λ max 272 nm and the amount of active was estimated from the formerly prepared calibration curve.[26]

4. Experimental wounding / wound creation

In the experiment, a total of 24 rats were used. The animals were randomly divided into four groups, with each group consisting of six rats, as detailed below:

Group 1: Negative control group (wounded rats)

Group II: Treated with standard drug povidine iodine (5%w/w)
Group III: Treated with 1% *Piper betle Linn.* leaf extract
Group IV: Test drug of optimized formulation (F4. emulgel of Ethanolic extract of *Piper betel Linn.*) was given to the wounded rats.

Excision wound model
i). Rats should be anesthetized in accordance with your proposed animal study. We recommend injecting a ketamine combination intraperitoneally. It provides surgical anaesthesia for roughly twenty minutes.

ii). Use a hair removal machine to remove the rats' dorsum hair.

iii). Apply the proper skin disinfectant to the surgical site. Our recommendation is 70% alcohol.

iv). After creating a sandwiched skinfold by folding and raising the dorsal skin cranially and caudally at the midline with the index and thumb fingers, position the animal laterally and press down with the 5-mm diameter sterile biopsy punch to remove the two layers of skin completely and create symmetrical full-thickness excisional wounds.

v). Place the animal in a warm location following surgery and keep an eye on its anaesthetic recovery. Place the animal back in its usual housing after it has fully healed. Cage each one separately.

vi).The Emulgel was applied topically once daily from the day of creation of excision wound, till the wound was completely healed. Control groups received vehicle (simple Emulgel base) topically. Group II were treated with the standard drug which is available in market. Group III were treated with the *Piper betle Linn.* leaf extract and Group IV were treated with optimized formulation twice a day. The wound areas were measured on 0^{th}, 3^{rd}, 6^{th}, 9^{th}, 12^{th}, 15^{th} and 18^{th} day, until the wound was healed for the experimental rats.

Wound closure monitoring after surgery
The animals are put to sleep as previously mentioned, and until the lesions have completely closed, the wound area is examined every two to three days. By using tracing paper, measure the wound size and determine the wound by applying the formula:

$$\frac{\text{Initial wound size (area)-specific wound size}}{\text{Initial wound size}} \times 100$$

RESULTS AND DISSCUSSION

Preparation of extract
The *Piper betle Linn.* leaves were subjected to Soxhlet extraction using ethanol as solvent, yielding a dark green crude extract rich in phytoconstituents. The obtained dry extract served as a stable and concentrated source of bioactive compounds for further formulation studies.

Phytochemical screening of extract
Table 3: Phytochemical screening of *Piper betle Linn.* ethanolic extract.

Sl. No	Constituents	Inference
1	Carbohydrates	+ve
2	Proteins	+ve
3	Alkaloids	+ve
4	Glycosides	+ve
5	Terpenes	+ve
6	Steroids	+ve
7	Flavonoids	+ve
8	Tannins	+ve
9	Saponins	+ve
10	Phenolics	+ve

i. Preformulation studies
a) Organoleptic evaluation
The organoleptic evaluation of *Piper betle Linn.* extract included assessments of its general characteristics, odor, and color. It was observed that *Piper betle Linn.* extract is Characteristic, strong aromatic odor, exhibiting Bitter, pungent taste and appears as a blackish-green amorphous semi solid.

b) Solubility Study
Solubility study of *Piper betle Linn.* extract in different solvents revealed that it is freely soluble in Ethanol, Methanol, DMSO and Coconut oil, soluble in Liquid paraffin, Slightly Soluble in Distilled water and Glycerol but insoluble in IPM.

c) Determination of λ max of *Piper betle Linn.* extract
A UV spectrophotometric analysis established that the maximum absorption wavelength (λ max) for *Piper betle Linn.* extract in ethanol is 272 nm, as illustrated in Fig.1. The obtained peak closely matched the values reported in the literature. This wavelength was chosen for further analysis.

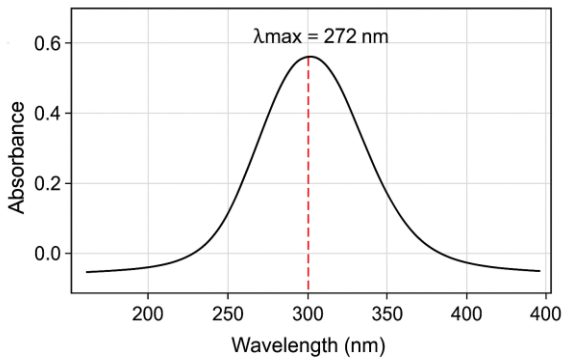


Figure no 1: Absorption maxima of *Piper betle Linn.* extract in ethanol.

d) Standard Calibration plot
A extract solution with concentrations ranging from 2 µg/ml to 10 µg/ml was prepared using ethanol, and

absorbance was measured using a UV spectrophotometer at the absorption maximum (λ max) of 272 nm against a blank (ethanol). A calibration curve was then constructed by plotting concentration on the x-axis and absorbance on the y-axis.

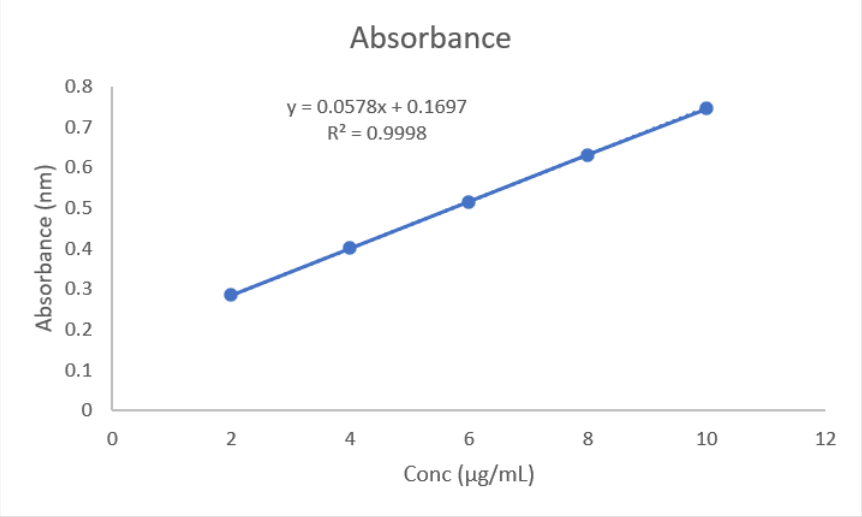


Figure no 2: Standard Calibration curve of *Piper betle Linn.* Extract.

The obtained absorbance data is plotted against the concentration of drug solution. Absorbance value remained linear and obeyed Beer's Lamberts law in the range of 0-10µg/ml with the slope value as y = 0.0578x + 0.1697 and R^2 value of 0.9998. The values of the absorbance at different conc (µg/ml) in ethanol are given in the table no.8 and the standard plot is shown in fig 2.

e) Compatibility Studies

Fourier transform infrared spectroscopy

The FTIR analysis revealed characteristic peaks of *Piper betle* Linn. extract, which showed slight shifts and intensity changes in the emulgel formulation. The broad O–H band shift indicated hydrogen bonding with the polymer matrix, while the disappearance of alkane C–H bands suggested masking within the gel. Shifts in C=O and C=C bands confirmed physical interactions and stabilization of the extract within the formulation. The observed variations in C–O and aromatic bands further supported molecular entrapment without chemical incompatibility. Overall, the results confirm good compatibility and stable incorporation of the extract into the emulgel base.

ii) Formulation development

a) Preparation of Emulsion and Stability

- **Selection of Oil phase**

Coconut oil was chosen as the oil phase in the emulsion formulation due to its excellent solubilizing property.

Comparative studies with other potential oil phases such as liquid paraffin, sesame oil, sunflower oil indicated that Coconut oil offered superior results in terms of drug solubility, justifying its selection as the preferred oil phase for this formulation.

- **Selection of Emulsifiers**

The required Hydrophilic Lipophilic balance (RHLB) for coconut oil was determined to be 8. To achieve this, a blend Span 80 (RHLB= 4.3) and Tween80 (RHLB= 15) was selected to create a stable emulsion.

- **Emulsion preparation**

Emulsions were prepared using the calculated blend of 34.57% Tween 80 and 65.42% in the ratio.

Span 80 with Smix concentrations ranging from 0.5% to 5%. The stability of these emulsions is then assessed, leading to the selection of a single concentration for further formulation batches.

- **Optimization of Emulsion**

Figure 3 presents The ternary phase diagram illustrates the relationship between Smix, oil, and water components in the emulsion system. The diagram with a larger emulsion region was selected for emulsion formulation, and ternary plots were constructed using Ternaryplot.com. Based on visual assessment, the mixture comprised approximately 80% water, 15% oil, and 5% Smix. The single data point positioned near the lower right corner of the triangle indicates a water-dominant composition, representing the optimal formulation identified through aqueous titration. This placement corresponds to a highly diluted oil-in-water (O/W) emulsion, aligning with the objective of achieving a stable emulsion with minimal surfactant concentration. The optimized ratio for the emulgel was finalized based on phase separation, creaming, and overall visual stability.

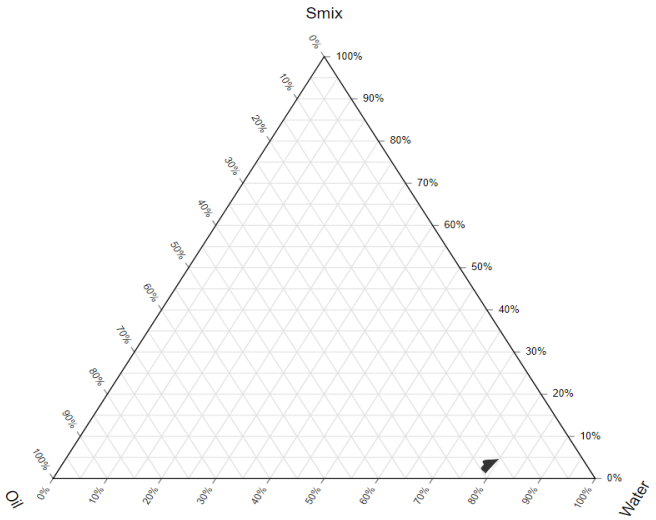


Figure no 3: Ternary plot.

b) Preparation of Emulgel

The optimized emulsion containing *Piper betle Linn.* extract was incorporated into gel base at various concentrations, maintaining a 1:1 ratio of emulsion to gel. Following the procedure outlined in the methodology section, a total of six emulgel formulations were developed.

These formulations comprised four variations using Carbopol 940 as gelling agent, each at different concentrations.

iii) Characterization of *Piper betle Linn.* extract emulgel

a) Physical Parameters of Prepared Formulations

The formulations were evaluated for their color, homogeneity and consistency.

Formulation F1, F2 and F3 were fluid due to the presence of low carbopol concentrations. Formulation F5 and F6 was thick pale yellow in color due to higher carbopol concentration. Formulation F4 which had 2% carbopol was Pale yellow creamy in appearance.

The physical appearances of all the formulations were found to be homogenous and consistent. No phase separation was observed in any of the formulated emulgels.

b) Microscopic study

The photo microscopic evaluations showed the presence of spherical globules, which revealed a uniform dispersion of oil droplets within the gel matrix formation of emulsion in gel base. This structure is consistent with a stable emulgel formulation and suggests good potential for controlled release of *Piper betle Linn.* extract.

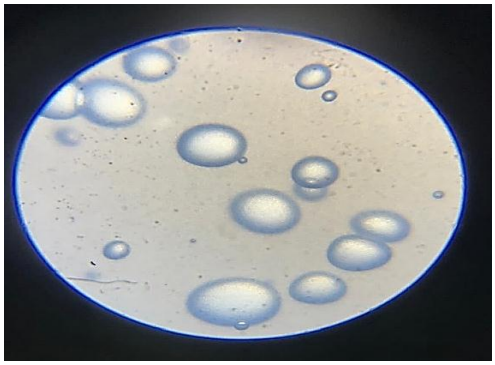


Figure 4: Emulgel formulated with Carbopol 940.

c) Gel strength

The gel strength was directly correlated with the concentration of Carbopol 940. The penetration time indicating gel strength, increased with increasing concentrations of carbopol 940. Carbopol 940 consistently exhibited longer penetration time, indicating stronger gel formation. Carbopol 940 was consistently more effective in forming strong gels compared to others, likely due to the formation of a more interconnected gel structure. Table 4 presents the data on gel strength.

Table no. 4: Gel Strength.

Gelling agent	Concentration (%w/w)	Penetration Time (s)
Carbopol 940	0.5	25 ± 1
	01	38 ± 2
	1.5	52 ± 1
	02	74 ± 3
	2.5	92 ± 2
	03	110 ± 3

d) Drug content determination

The average percentage drug content present in 1 gm of emulgel formulation is displayed in the table no 5. It was noted that the extract was evenly distributed throughout the formulation. The drug content of the formulations was found to be within the specified range of 95% - 105%

Table no. 5: Drug content.

Formulation	Drug content (%) (Mean ± SD)
F1	96.5± 0.215
F2	97.0 ± 0.126
F3	97.5± 0.252
F4	98.2 ± 0.418
F5	97.4 ± 0.596
F6	96.8± 0.180

All values represented as mean ± standard deviation (n=3)

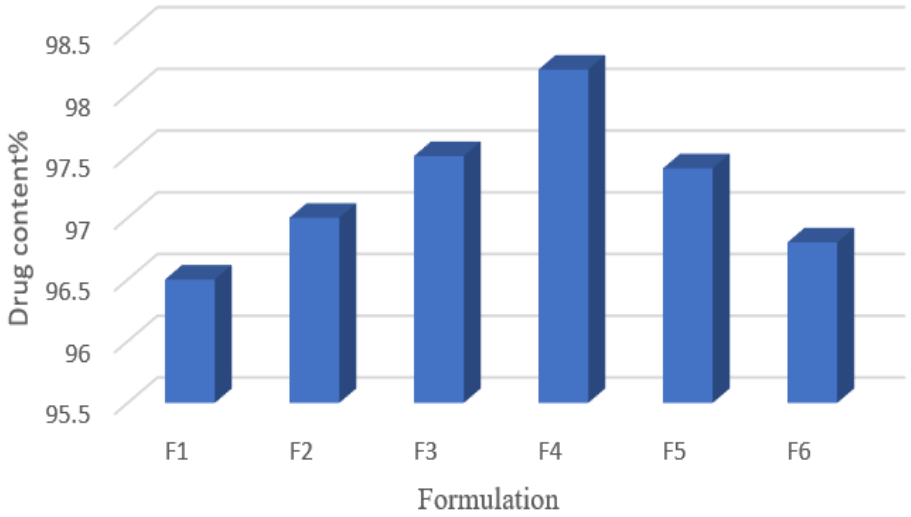


Figure 5: Graphical representation of Drug content.

All formulations showed drug content within the pharmacopeial limits (95–105%), with F4 exhibiting the highest value (98.2%), indicating better uniformity and compliance with standards.

e) Determination of pH

The pH values of all prepared emulgel formulations ranged from 5.70 to 7.01 as indicated in the Table 6. These levels are deemed acceptable, as they minimize the risk of skin irritation, considering the average pH of adult skin is 5.5.

Table 6: pH of the formulations F1 – F6.

Formulation	pH (Mean ± SD)
F1	6.44 ± 0.02
F2	6.12 ± 0.03
F3	5.87 ± 0.02
F4	5.70 ± 0.07
F5	7.014 ± 0.14
F6	6.64 ± 0.01

All values represented as mean ± standard deviation (n=3)

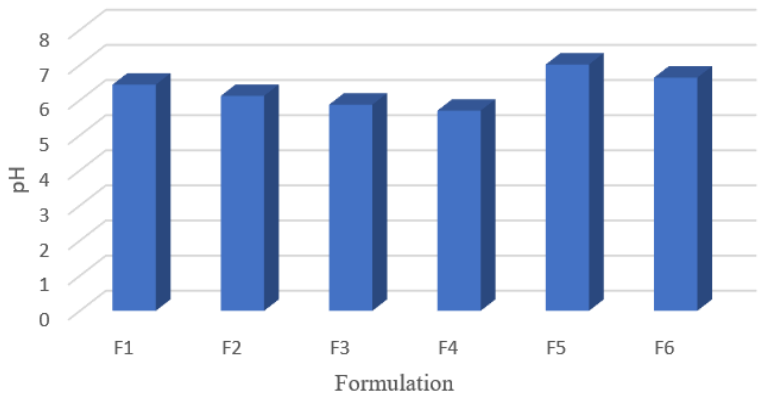


Figure 6: Graph of pH.

All formulations exhibited skin-compatible pH values, but F4 (pH 5.70) was found to be the closest to the natural skin pH of 5.5 when compared with the standard, establishing F4 as the most appropriate formulation for safe topical use.

f) Spreadability studies

The spreadability of *Piper betle Linn.* extract emulgel formulation following the spreadability test was found to range from 9.5g.cm/min to 12.8g.cm/min for the formulations F1-F6 and the data are given in the Table 7**.**

By taking the data into consideration, it was observed that concentration of gelling agent makes the difference in the spreadability.

The spreadability of the formulations was influenced by polymer concentration and viscosity. A higher polymer concentration led to reduced spreadability. All formulations exhibited satisfactory spreadability properties. Emulgels prepared with low concentration of Carbopol 940 i.e. F1, F2 and F3 belonged to soft gel category, having more spreadability values. The formulations F4 belonged to semi stiff category, whereas F5and F6 prepared with higher concentration of Carbopol belonged to very stiff category. With Increase in gelling agent concentration in formulation, the spreadability of formulations decreases. The values of spreadability indicate that the emulgel is easily spreadable with minimal shear.

Table 7: Data for Spreadability.

Formulation	Spreadability (gm.cm/sec)
F1	12.5± 0.05
F2	10.83 ± 0.07
F3	9.67± 0.14
F4	8.67 ± 0.12
F5	7.5 ± 0.03
F6	6.67 ± 0.06

All values represented as mean ± standard deviation (n=3)

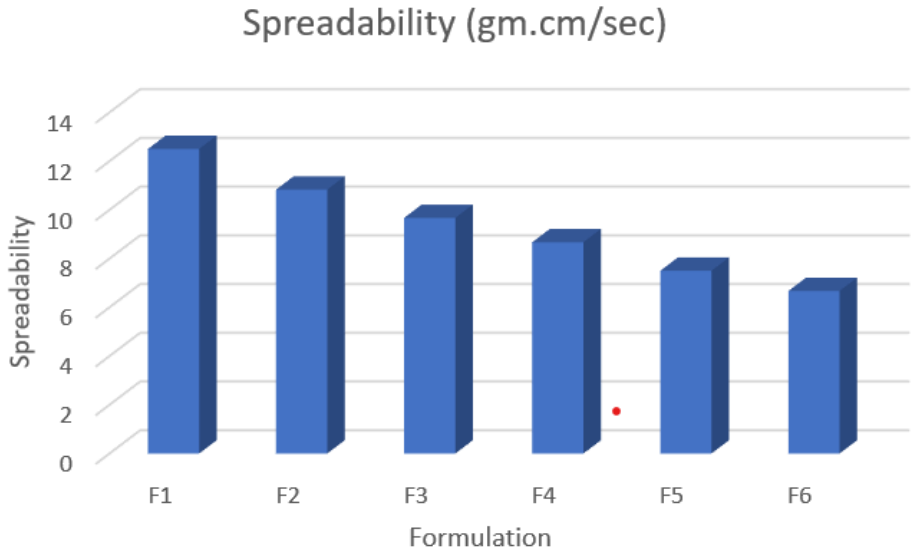


Figure 7: Graph of Spreadability.

All formulations exhibited satisfactory spreadability (6.67–12.5 g·cm/sec) within acceptable limits (standard: 5–15 g·cm/sec), with F4 showing the highest value (8.67 g·cm/sec), indicating superior ease of application and optimal polymer concentration.

g) Viscosity determination

Viscosity of the emulgel formulation F4 was determined using a Brookfield RV viscometer. The emulgel was rotated at different speed (rpm) with spindle no 7.

Table 8: Data for Viscosity determination.

Formulation	Viscosity (cP), 50 rpm
F1	3,270 ± 25.10
F2	6,830 ± 21.5
F3	11,286 ± 17.10
F4	14,150 ± 16.20
F5	19,613± 14.10
F6	25,545± 18.10

All values represented as mean ± standard deviation (n=3)

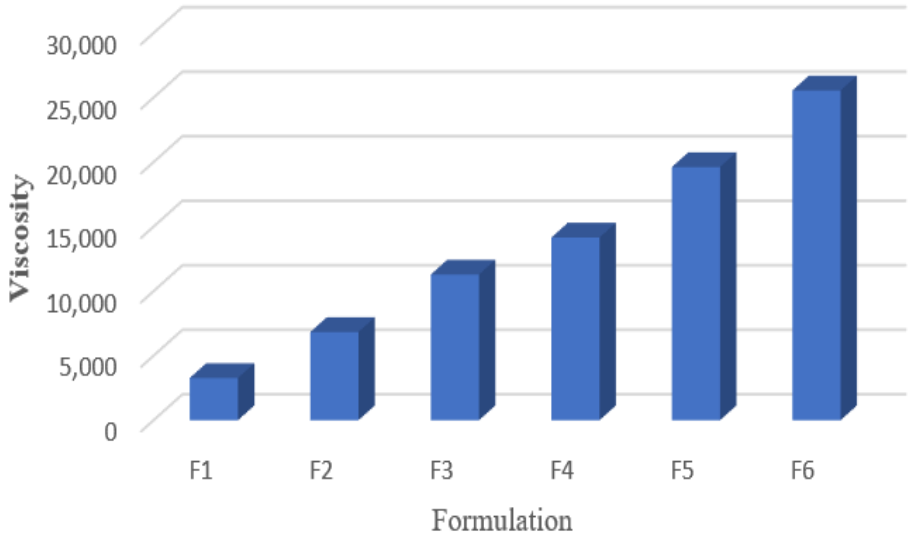


Figure 8: Graphical representation of Viscosity.

The viscosity of formulations increased with rising Carbopol 940 concentration (3,200–25,500 cP), remaining within the standard topical emulgel range (3,000–30,000 cP); F4 (14,150 cP) exhibited optimal viscosity, ensuring both good spreadability and structural stability.

h) Swelling Index

The swelling index of a formulation F4 were tested. It was observed that Emulgel formulated with Carbopol-940 (F4) as the gelling agent demonstrated significantly higher swelling indices. The superior swelling behavior of Carbopol 940 is an important factor in the performance and drug release characteristics of emulgel formulations. The results of the swelling index are shown below.

Table 9: Swelling Index of F4.

Time (hr)	Swelling Index (%) (Mean ± SD)
0.5	19.3 ± 0.25
1	36.4 ± 0.48
1.5	49.8 ± 0.62
2	59.1 ± 0.98
2.5	72.4 ± 0.39
3	88.2 ± 0.87
3.5	92.6 ± 0.66
4	100.2 ± 0.85

All values represented as mean ± standard deviation (n=3)

The swelling index results confirmed that formulation F4 with Carbopol-940 showed the highest swelling capacity (100.2% at 4 hrs), outperforming the standard, there by establishing F4 as the best formulation for improved drug release and stability of emulgel.

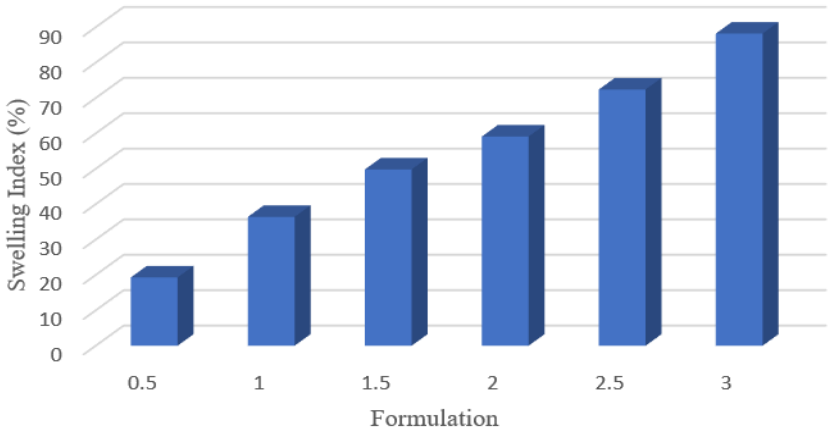


Figure 9: Graphical depiction of the Swelling Index.

i) *In vitro* Drug release

The therapeutic efficacy of any drug depends upon the drug release from pharmaceutical dosage forms. After formulation development, the formulations F1, F2, F3 and F4, F5, F6 were assessed for *in vitro* drug release profile from 0.5 hour to 8 hours. The percentage release of *Piper betle Linn.* extract from optimized emulgel formulation F4 was found to be 92.3% after 8 hours. The *In vitro* drug release data and percentage release of drug from the formulations are shown in the Table 10 and 11, Figure 10 and 11 respectively.

Table no. 10:* Invitro *drug release data of F1– F3.

Time (hr)	*Invitro* drug release (%) (Mean ± SD)		
	F1 (%)	F2 (%)	F3 (%)
0.5	9.92 ± 0.6	10.55 ± 0.6	13.84 ± 0.6
1	14.34 ± 0.7	17.14 ± 0.7	20.05 ± 0.7
2	26.86 ± 0.9	29.65 ± 0.9	36.42 ± 0.9
3	36.14 ± 0.8	42.81 ± 0.8	51.62 ± 0.8
4	46.45 ± 0.7	51.25 ± 0.7	58.38 ± 0.7
5	55.58 ± 0.6	61.53 ± 0.6	67.82 ± 0.6
6	63.64 ± 0.8	70.37 ± 0.8	74.19 ± 0.8
7	68.84 ± 0.7	74.21 ± 0.7	80.03 ± 0.7
8	70.06 ± 0.6	79.46 ± 0.6	84.91 ± 0.6

All values represented as mean ± standard deviation (n=3)

Table no 11:* Invitro *drug release data of F4– F6.

Time (hr)	*Invitro* drug release (%) (Mean ± SD)		
	F4 (%)	F5 (%)	F6 (%)
0.5	15.86 ± 0.6	10.91 ± 0.6	8.29 ± 0.6
1	25.53 ± 0.7	20.35 ± 0.7	16.70 ± 0.7
2	45.29 ± 0.9	34.23 ± 0.9	29.75 ± 0.9
3	60.46 ± 0.8	49.45 ± 0.8	41.05 ± 0.8
4	70.81 ± 0.7	57.31 ± 0.7	49.68 ± 0.7
5	77.70 ± 0.6	66.91 ± 0.6	58.05 ± 0.6
6	83.24 ± 0.8	72.02 ± 0.8	65.81 ± 0.8
7	87.18 ± 0.7	77.90 ± 0.7	70.51 ± 0.7
8	92.36 ± 0.6	81.57 ± 0.6	73.67 ± 0.6

All values represented as mean ± standard deviation (n=3)

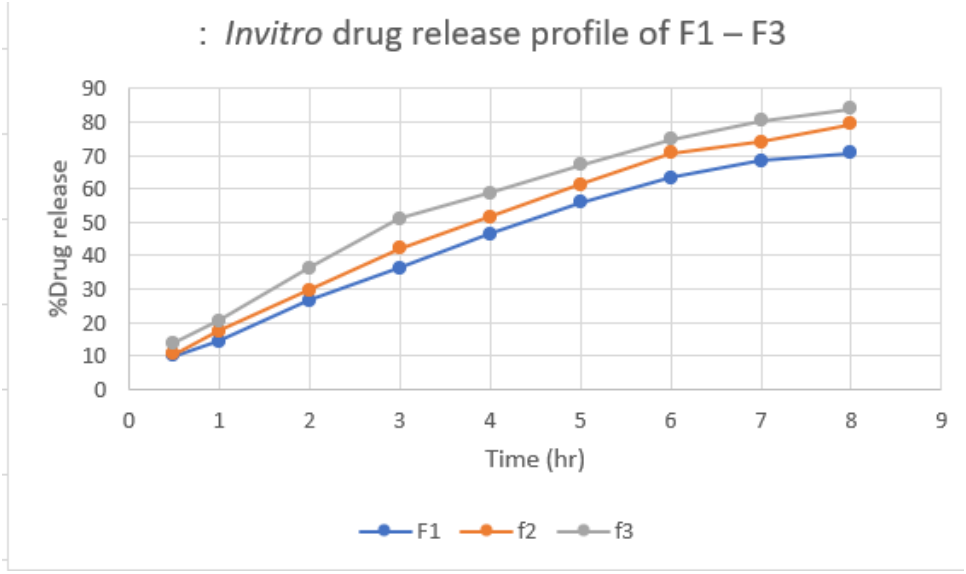


Figure no. 10: *Invitro* drug release profile of F1 – F3 formulation.

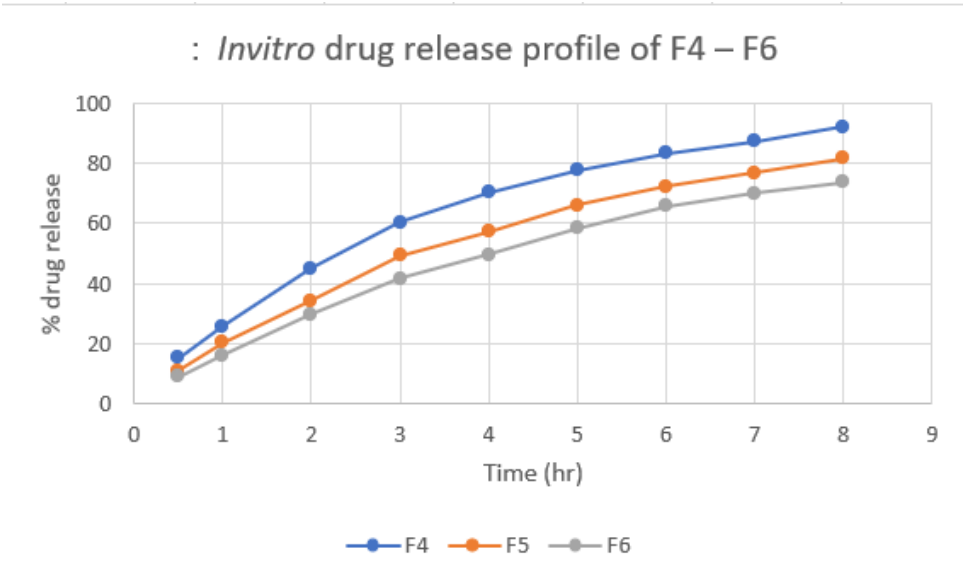


Figure no 11: *Invitro* drug release profile of F4 – F6 formulation.

Ex-vivo Wound Healing Activity Assessment

The animal study was initiated after obtaining IAEC approval (**SVCP/ IAEC/R04/2025**). A total of 24 Wistar albino rats were used and randomly divided into four groups, each consisting of six rats.

The effect of the ethanolic extract of *Piper betle Linn*. and its emulgel formulation on wound healing was evaluated using the excision wound model. The wound contraction ability of the standard and test groups was found to be significantly higher than that of the control group (Table 13 and Fig. 12,13). The percentage of wound closure was determined by measuring changes in wound area at fixed intervals of time.

Rats treated with the optimized emulgel formulation (Group IV) exhibited wound healing activity comparable to those treated with the standard drug (povidone iodine 5% w/w). The control group showed gradual but slower wound healing compared to the test and standard groups.

All four groups showed a progressive reduction in wound area over the experimental period. By the 18th day, the control group achieved 97% wound closure, the *Piper betle Linn.* leaf extract group showed 98.5%, and the standard group reached complete (100%) closure. The emulgel-treated group demonstrated 100% closure by the 15th day, indicating faster and more effective wound healing.

These findings confirm that the ethanolic extract of *Piper betle* Linn., when formulated as an emulgel (F4), significantly enhances wound contraction and healing, showing efficacy comparable to the standard povidone iodine ointment.

Table no. 13: *Ex-vivo* wound healing activity assessment.

Groups	Day 0	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18
Control	0.00%	7.7±0.31%	22.67±0.263%	40.67±0.288%	67.92±0.25%	87.65±0.369%	97.45±45%
Standard	0.00%	17.55±0.351%	45.37±0.287%	62.52±0.15%	81.45±0.369%	96.92±0.292%	100%
***Piper betle Linn.* leaf extract**	0.00%	11.3 ± 0.294%	22.5 ± 0.258%	51.02 ± 0.189%	82.37 ± 0.327%	91.25 ± 0.235%	98.45% ± 0.238
***Piper betle Linn.* leaf extract Emulgel**	0.00%	12.32 ± 0.152%	35.42 ± 0.247%	63.55 ± 0.387%	90.92 ± 0.242%	100%	Nill

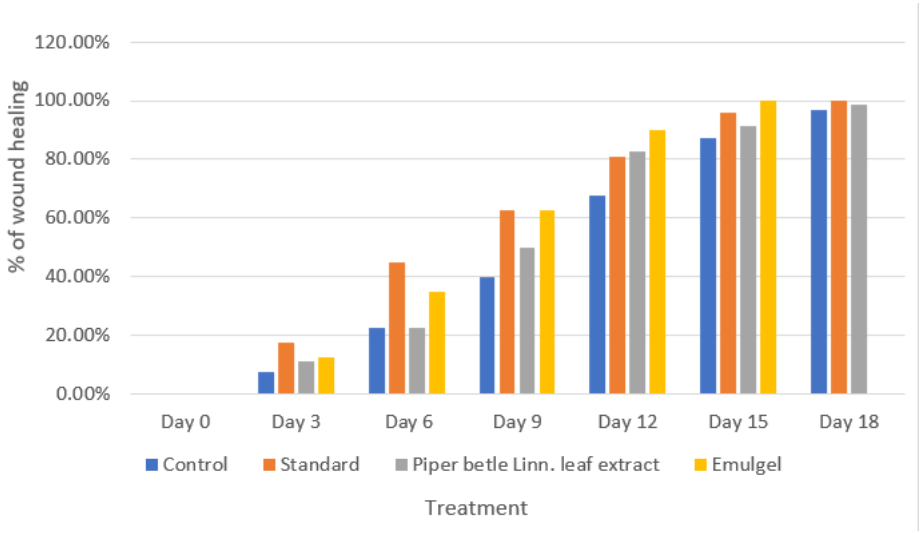
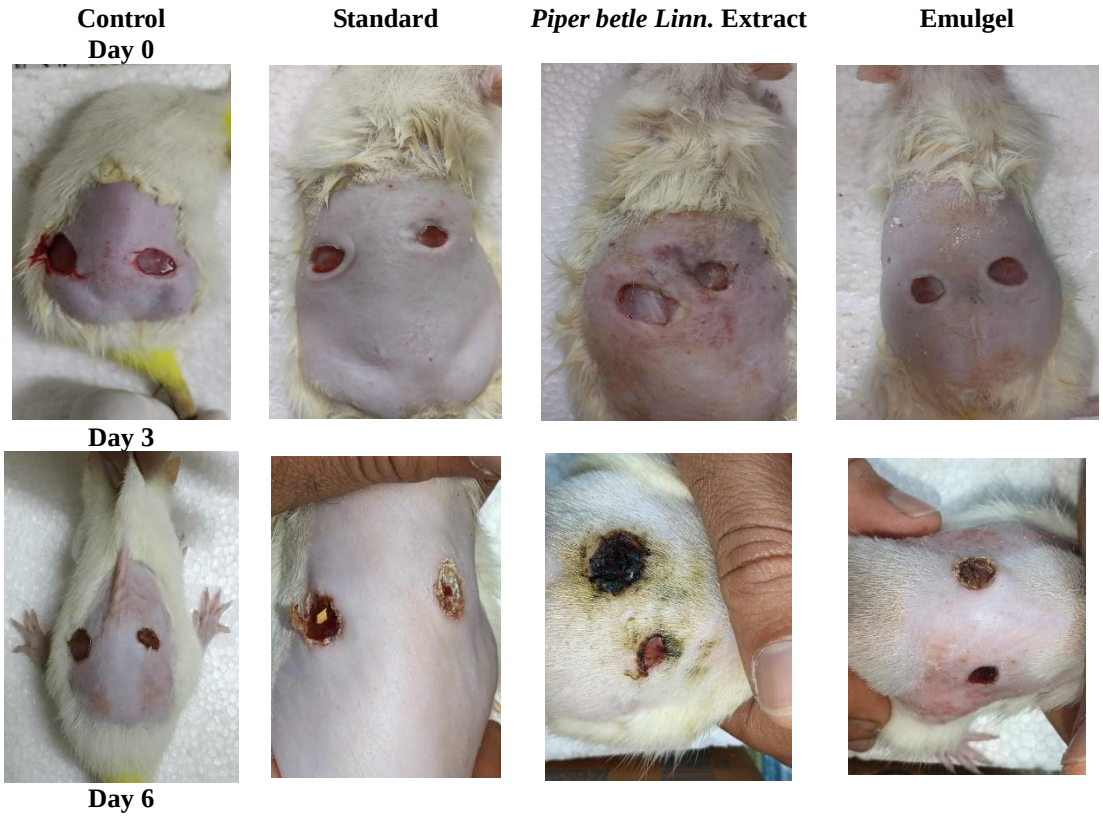


Figure no. 12: Graphical depiction of the *Ex-vivo* wound healing activity.

4. Excision wound model



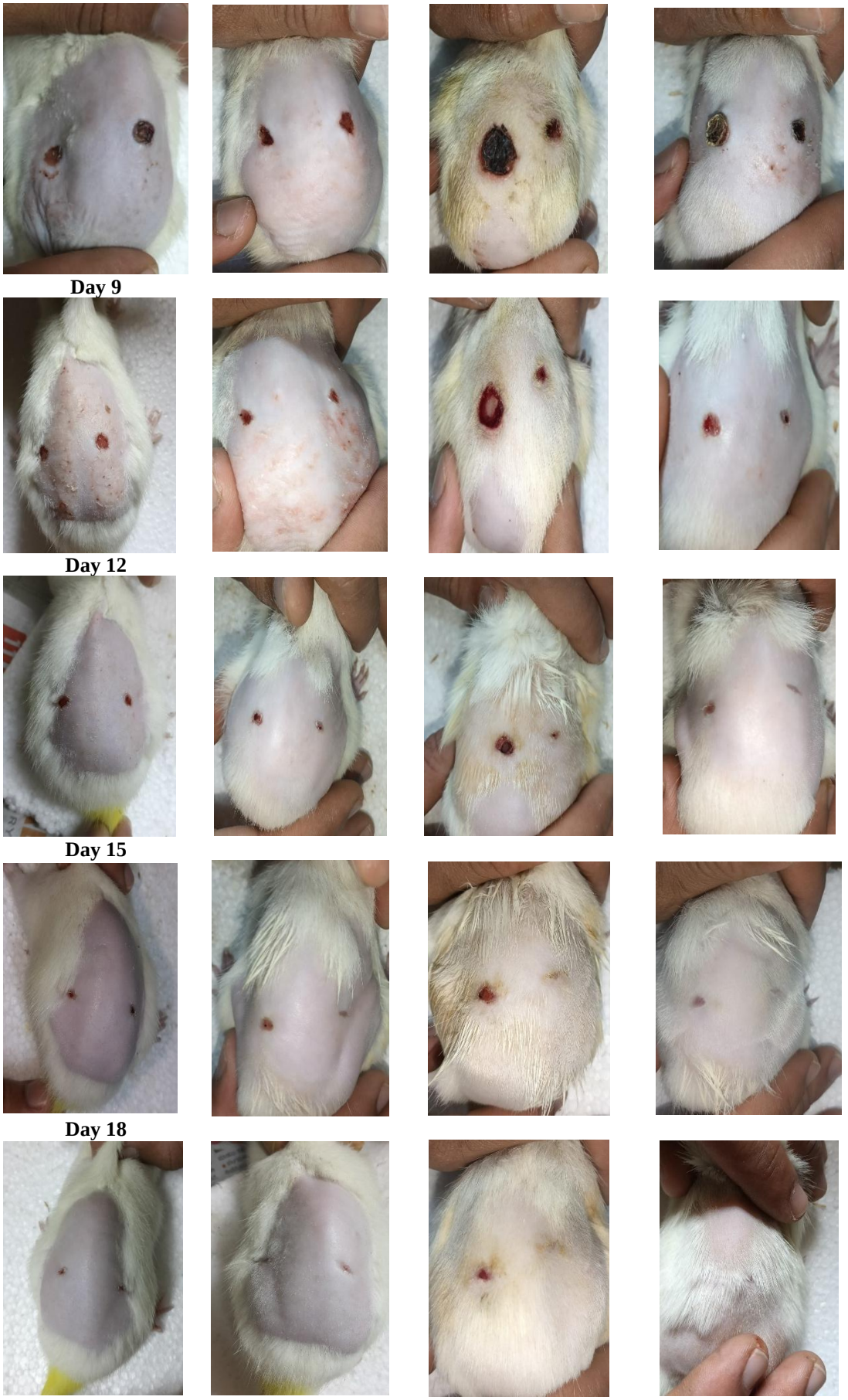


Figure no. 13: Wound healing images.

CONCLUSION

The present research focused on the development and evaluation of a topical emulgel containing ethanolic extract of *Piper betle Linn*. leaf for wound healing applications. Qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, proteins, and carbohydrates in the extract. Preformulation studies confirmed favorable physicochemical properties, including strong aromatic odor, bitter-pungent taste, blackish-green semi-solid state, and solubility profile in various solvents. UV spectrophotometric analysis identified λmax at 272 nm with excellent linearity, providing a reliable method for drug quantification. FTIR studies confirmed drug–excipient compatibility, and coconut oil with a calculated blend of Tween 80 (34.57%) and Span 80 (65.42%) ensured stable emulsification. Six emulgel formulations (F1–F6) were prepared using Carbopol 940; all were stable, homogeneous, and free from phase separation. Among them, F4 (2% Carbopol 940) showed optimal drug content (98.2%), skin-compatible pH (5.70), spreadability (8.67 g·cm/sec), viscosity (14,150 cP), uniform globule dispersion, and favorable swelling (100.2% in 4 h). In vitro release demonstrated sustained drug release, with F4 achieving 92.3% at 8 hours. Pharmacological evaluation confirmed that the emulgel was safe, non-sensitizing, and significantly enhanced wound healing compared to crude extract, showing efficacy comparable to the standard drug. Thus, F4 was identified as the most promising formulation, offering improved bioavailability, sustained release, and superior dermal penetration for effective wound management. It was ultimately concluded that formulation f4 emerged as the most promising, exhibiting superior physicochemical properties and greater pharmacological activity than the other formulations. Further research can be undertaken to enhance and optimize its performance.

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