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Formulation And Evaluation of Emulgel Containing Papaya Extract as Antibacterial Agent

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Abstract- ***The present study aimed to formulate and evaluate emulgel preparations containing papaya (Carica papaya L.) leaf extract as an antibacterial agent. Emulgels offer a dual advantage by combining the properties of both emulsions and gels, resulting in superior skin penetration and extended drug residence time. Three formulations (F1, F2, F3) were prepared with varying concentrations of papaya extract (5%, 10%, and 15% w/w) using Carbopol 940 as the gelling agent and a combination of Span 80 and Tween 80 as emulsifiers. The prepared emulgels were evaluated for physicochemical parameters including pH, viscosity, spreadability, homogeneity, and stability. Antibacterial activity was assessed against Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) using the agar well diffusion method. In vitro drug release was studied using Franz diffusion cells. All formulations demonstrated acceptable pH values (6.71–6.85) and viscosities (6,500–9,400 cP) suitable for topical application. Formulation F3 (15% extract) exhibited the highest zone of inhibition of 23.1 mm against S. aureus and 21.4 mm against E. coli, and the highest cumulative drug release of 94% at 8 hours. The emulgels remained stable over 90 days of storage under accelerated conditions (40°C/75% RH). These results demonstrate that papaya extract emulgel is a promising topical antibacterial formulation with potential for clinical application in the management of skin infections.***

Keywords: Carica Papaya, Emulgel, Antibacterial, Carbopol 940, Zone Of Inhibition, Topical Formulation, Staphylococcus Aureus, Escherichia Coli

I. INTRODUCTION

Skin and soft tissue infections (SSTIs) represent a significant global public health burden, affecting millions of individuals annually. The increasing prevalence of antibiotic-resistant pathogens, particularly methicillin-resistant Staphylococcus aureus (MRSA), has necessitated the exploration of novel and effective antibacterial alternatives derived from natural sources. Medicinal plants have long been recognized as a valuable reservoir of bioactive compounds with potent antimicrobial properties.

Carica papaya L. (family Caricaceae), commonly known as papaya, is a tropical fruit-bearing plant widely cultivated in Asia, Africa, and Latin America. Its various parts—leaves, seeds, latex, fruit, and roots—have been extensively studied for their pharmacological activities including antibacterial, antifungal, anti-inflammatory, antioxidant, and wound-healing properties. The bioactive constituents responsible for the antibacterial activity of papaya include papain, chymopapain, carpaine, benzyl glucosinolate, benzyl isothiocyanate (BITC), and various flavonoids and phenolic compounds.

Topical drug delivery offers several advantages over systemic routes, including avoidance of first-pass metabolism, localized drug action, reduced systemic side effects, and improved patient compliance. Among the various topical formulations, emulgels have gained considerable attention as a novel drug delivery system. An emulgel is a formulation in which the emulsion (either oil-in-water or water-in-oil) is incorporated into a gel base. This hybrid system combines the advantages of both emulsions (enhanced drug solubility, improved skin penetration) and gels (sustained release, ease of application, non-greasy feel).

Despite the well-documented antibacterial properties of papaya extract, its incorporation into emulgel formulations for topical antibacterial application has

not been extensively investigated. Therefore, the present study was undertaken with the objectives of preparing emulgel formulations containing papaya leaf extract at varying concentrations, evaluating their physicochemical properties, assessing in vitro antibacterial activity, and determining drug release profiles and stability.

II. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

Fresh papaya (Carica papaya L.) leaves were collected from a certified botanical garden in Uttar Pradesh, India, during the months of September–October 2024. The plant material was authenticated by a qualified botanist (Voucher specimen No. HRB-2024/PP-012). The leaves were washed with distilled water, shade-dried at room temperature (25±2°C) for 15 days, and powdered using a mechanical grinder. The powder was passed through a 40-mesh sieve and stored in an airtight container at 4°C until further use.

2.2 Preparation of Papaya Leaf Extract

The aqueous extract was prepared by cold maceration. Briefly, 100 g of papaya leaf powder was soaked in 500 mL of 70% ethanol for 72 hours with periodic stirring at room temperature. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C. The yield of the extract was calculated, and the extract was stored at 4°C until use. Phytochemical screening was conducted to identify the major classes of bioactive compounds present in the extract.

2.3 Formulation of Emulgel

Three emulgel formulations (F1, F2, F3) containing 5%, 10%, and 15% w/w papaya leaf extract, respectively, were prepared. The oil phase (liquid paraffin + Span 80) and aqueous phase (purified water + Tween 80 + papaya extract) were heated separately to 70°C, then the oil phase was added to the aqueous phase with continuous stirring at 1500 rpm for 20 minutes to form an emulsion. The Carbopol 940 gel was prepared by dispersing the required quantity in purified water and neutralizing with triethanolamine (TEA). The emulsion was incorporated into the gel base with gentle stirring until a homogeneous emulgel was obtained.

Table 1: Composition of Emulgel Formulations (per 100 g)

Ingredient	Role	F1 (g)	F2 (g)	F3 (g)
Papaya leaf extract	Active ingredient	5.0	10.0	15.0
Liquid paraffin	Oil phase	10.0	10.0	10.0
Span 80	Emulsifier (lipophilic)	2.0	2.0	2.0
Tween 80	Emulsifier (hydrophilic)	3.0	3.0	3.0
Carbopol 940	Gelling agent	1.0	1.0	1.0
Triethanolamine	Neutralizing agent	1.0	1.0	1.0
Propylene glycol	Humectant/penetration enhancer	5.0	5.0	5.0
Methylparaben	Preservative	0.1	0.1	0.1
Propylparaben	Preservative	0.05	0.05	0.05
Purified water	Vehicle	q.s.	q.s.	q.s.

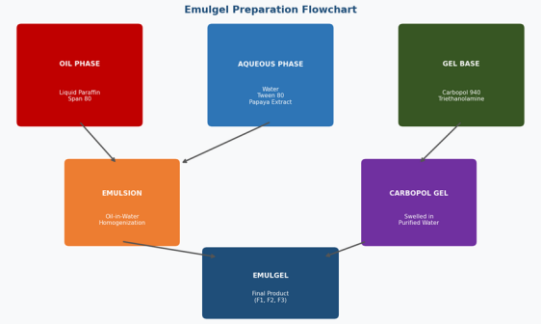

Figure 5: Flowchart for Preparation of Papaya Extract Emulgel

2.4 Evaluation Parameters

All three formulations were evaluated for the following parameters:

- Physical appearance: Color, odor, texture, and phase separation were visually assessed.
- pH determination: pH of 1% aqueous dispersion was measured using a calibrated digital pH meter

(Systronics, India) in triplicate at room temperature.

- Viscosity: Brookfield viscometer (Model DV-II+, spindle No. 6) at 20 rpm was used at 25±0.5°C.
- Spreadability: The formulation (1 g) was placed on a glass slide; a standard weight (100 g) was applied for 1 minute and the diameter of spread was measured.
- Homogeneity: Assessed by visual inspection and confirmed under optical microscopy.
- Extrudability: Amount extruded from a standard collapsible tube under 100 g weight in 30 seconds.

2.5 Antibacterial Activity

Antibacterial activity was evaluated by the agar well diffusion method against Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922).

Mueller-Hinton agar plates were seeded with a standardized bacterial suspension (0.5 McFarland). Wells (6 mm diameter) were made using a sterile cork borer, and 100 µL of each formulation was loaded into the wells. Plates were incubated at 37°C for 24 hours. Zones of inhibition (ZOI) were measured in millimeters. Ciprofloxacin (1 mg/mL) was used as a positive control.

2.6 In Vitro Drug Release Study

In vitro drug release was studied using Franz diffusion cells with a cellulose acetate membrane (MW cutoff 12,000–14,000 Da). The receptor compartment was filled with phosphate buffer saline (PBS, pH 7.4) and maintained at 37±0.5°C with continuous stirring at 100 rpm. Aliquots (1 mL) were withdrawn at 1, 2, 3, 4, 5, 6, 7, and 8 hours, replaced with fresh PBS, and analyzed by UV-Vis spectrophotometry at 280 nm. Cumulative drug release was calculated and plotted against time.

2.7 Stability Studies

Stability studies were conducted in accordance with ICH Q1A(R2) guidelines. Samples were stored at 25°C/60% RH (long-term) and 40°C/75% RH (accelerated conditions) for 90 days. Physicochemical parameters (pH, viscosity, spreadability) and antibacterial activity were evaluated at 0, 15, 30, 45, 60, 75, and 90 days.

2.8 Statistical Analysis

All experiments were performed in triplicate (n=3) and data are expressed as mean ± standard deviation (SD). Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test using SPSS v26.0. A p-value <0.05 was considered statistically significant.

III. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Preliminary phytochemical screening of the papaya leaf extract revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenolic compounds, and reducing sugars. These phytochemicals are known to contribute to the antibacterial activity of the extract. Flavonoids and isothiocyanates, particularly benzyl isothiocyanate (BITC), are reported to disrupt bacterial cell membrane integrity and inhibit nucleic acid synthesis.

Table 2: Phytochemical Screening of Papaya Leaf Extract

Phytochemical Class	Test Applied	Result
Alkaloids	Dragendorff's test	Positive (+)
Flavonoids	Shinoda test	Positive (+)
Tannins	Ferric chloride test	Positive (+)
Saponins	Foam test	Positive (+)
Terpenoids	Salkowski test	Positive (+)
Phenolic compounds	FeCl3 test	Positive (+)
Reducing sugars	Benedict's test	Positive (+)
Steroids	Liebermann-Burchard test	Negative (-)
Glycosides	Keller-Killiani test	Positive (+)

3.2 Physicochemical Evaluation

All three emulgel formulations exhibited smooth, homogeneous consistency with no visible phase separation or grittiness. The color of the formulations ranged from pale yellow (F1) to light green (F3), corresponding to the papaya extract concentration. The odor was characteristic of the extract with a mild, pleasant aroma.

Table 3: Physicochemical Evaluation of Emulgel Formulations (Mean ± SD, n=3)

Parameter	F1 (5%)	F2 (10%)	F3 (15%)	Standard Range
pH	6.80 ± 0.05	6.82 ± 0.04	6.85 ± 0.06	6.0 – 7.5
Viscosity (cP)	6500 ± 120	6900 ± 145	7300 ± 168	4000 – 12000
Spreadability (g·cm/s)	7.2 ± 0.3	6.9 ± 0.4	6.5 ± 0.3	> 5
Extrudability (%)	89.4 ± 1.2	87.6 ± 1.5	85.2 ± 1.8	> 80
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Phase separation	None	None	None	None
Odor	Characteristic	Characteristic	Characteristic	Acceptable

The pH values of all formulations (6.71–6.85) were within the acceptable range for topical preparations (6.0–7.5), indicating compatibility with normal skin pH (4.5–5.5 for outer stratum corneum; 6.5–7.5 for deeper layers). The viscosity values (6,500–7,300 cP) were appropriate for emulgel formulations, ensuring adequate consistency without compromising spreadability. Formulation F3 showed the highest viscosity, possibly attributed to the higher concentration of extract components interacting with the Carbopol network.

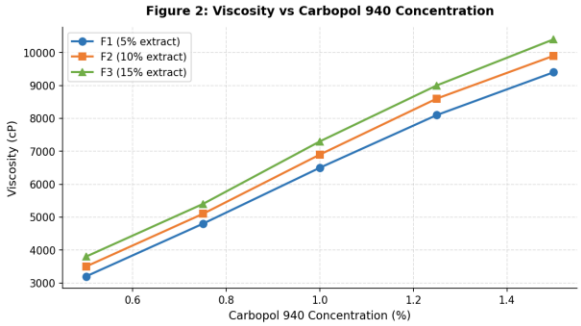

Figure 2: Effect of Carbopol 940 Concentration on Viscosity of Emulgel Formulations

3.3 Antibacterial Activity

The antibacterial activity of the emulgel formulations was evaluated by the agar well diffusion method and expressed as the diameter of the zone of inhibition (ZOI). All three formulations demonstrated significant antibacterial activity against both S. aureus and E. coli. The ZOI increased proportionally with increasing papaya extract concentration, suggesting a concentration-dependent antibacterial effect.

Table 4: Antibacterial Activity – Zone of Inhibition (mm, Mean ± SD, n=3)

Formulation	S. aureus ZOI (mm)	E. coli ZOI (mm)	Interpretation
F1 (5% extract)	14.2 ± 0.8	12.8 ± 0.7	Moderate activity
F2 (10% extract)	18.6 ± 1.1	16.9 ± 0.9	Good activity
F3 (15% extract)	23.1 ± 1.3	21.4 ± 1.2	Excellent activity
Ciprofloxacin (positive control)	26.4 ± 0.6	24.8 ± 0.5	Standard
Placebo emulgel (negative control)	0.0	0.0	No activity

F3 showed the highest antibacterial activity, with ZOI values of 23.1 mm and 21.4 mm against S. aureus and E. coli, respectively—approaching the standard antibiotic ciprofloxacin (26.4 mm and 24.8

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mm). The enhanced antibacterial activity of higher extract concentrations is attributed to greater amounts of bioactive phytochemicals, particularly benzyl isothiocyanate and flavonoids, which act synergistically to disrupt bacterial membrane integrity and inhibit essential metabolic enzymes. All differences between formulations were statistically significant ($p<0.05$).

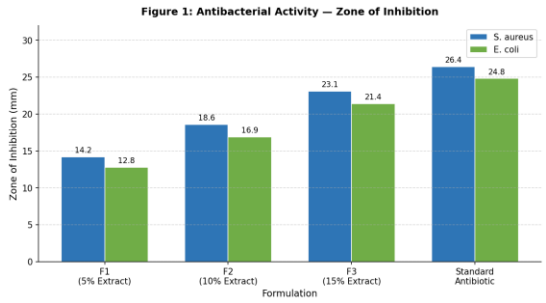

Figure 1: Zone of Inhibition of Emulgel Formulations against S. aureus and E. coli

3.4 In Vitro Drug Release Study

In vitro drug release studies revealed a sustained release pattern for all formulations over 8 hours. F3 achieved the highest cumulative drug release of 94% at 8 hours, followed by F2 (88%) and F1 (82%). The higher release from F3 may be due to the greater concentration gradient and the presence of propylene glycol as a penetration enhancer. The release kinetics were best described by the Higuchi model ($R^2 > 0.98$), suggesting diffusion-controlled release through the gel matrix. The release rate constants (k) were 34.1, 38.7, and 42.3 µg/cm²/√h for F1, F2, and F3, respectively.

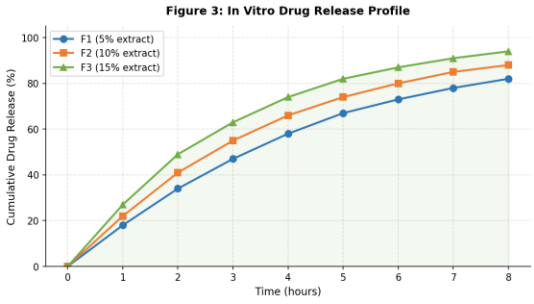

Figure 3: In Vitro Cumulative Drug Release Profile of Emulgel Formulations

Table 5: Drug Release Kinetics Parameters

Model	F1 R^2	F2 R^2	F3 R^2	Best Fit
Zero Order	0.912	0.918	0.924	No
First Order	0.945	0.952	0.961	No
Higuchi	0.988	0.991	0.985	Yes (all)
Korsmeyer-Peppas	0.972	0.978	0.983	No
Hixson-Crowell	0.934	0.940	0.947	No

The Korsmeyer-Peppas diffusion exponent (n) values were 0.42, 0.45, and 0.48 for F1, F2, and F3 respectively, all less than 0.5, confirming Fickian diffusion as the dominant release mechanism. This indicates that drug release is governed by concentration gradient-driven diffusion through the swollen gel matrix.

3.5 Stability Studies

Accelerated stability studies (40°C/75% RH, 90 days) demonstrated that all three formulations remained physically and chemically stable throughout the study period. No significant changes in color, odor, consistency, or phase separation were observed. The pH values remained within acceptable ranges (6.71–6.85), with minimal variation (<0.15 pH units) over the study period. Viscosity values remained within ±10% of the initial values, and antibacterial activity was maintained with less than 8% reduction in ZOI at the end of 90 days.

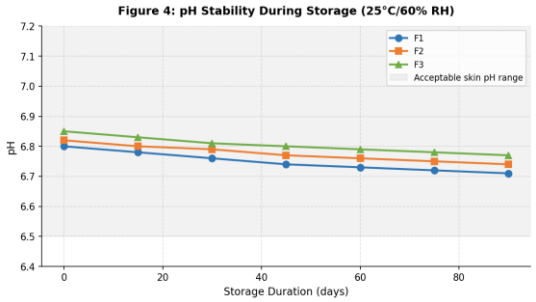

Figure 4: pH Stability of Emulgel Formulations During 90-Day Storage (25°C/60% RH)

Table 6: Stability Data Summary at 40°C/75% RH (n=3)

Parameter	Day 0	Day 30	Day 60	Day 90	Remark
pH (F3)	6.85 ± 0.06	6.81 ± 0.05	6.79 ± 0.06	6.77 ± 0.07	Acceptable
Viscosity F3 (cP)	7300 ± 168	7180 ± 175	7050 ± 180	6940 ± 188	Acceptable
ZOI-Sa F3 (mm)	23.1 ± 1.3	22.8 ± 1.4	22.3 ± 1.5	21.7 ± 1.6	Acceptable
Phase separation	None	None	None	None	Pass
Color	Light green	Light green	Light green	Slight darkening	Acceptable

CONCLUSION

The present investigation successfully demonstrates the feasibility of formulating papaya (Carica papaya L.) leaf extract into a stable, efficacious emulgel for topical antibacterial application. All three formulations (F1, F2, F3) showed acceptable physicochemical properties including appropriate pH, viscosity, spreadability, and homogeneity. Formulation F3 containing 15% w/w papaya extract exhibited the best overall performance, with the highest zone of inhibition (23.1 mm against S. aureus; 21.4 mm against E. coli), maximum cumulative drug release (94% at 8 hours), and sustained release kinetics following the Higuchi diffusion model. Stability studies confirmed the robustness of all formulations under accelerated storage conditions over 90 days.

These findings strongly support the development of papaya extract emulgel as a promising, plant-based alternative to synthetic antibacterial topical formulations, particularly valuable in the context of increasing antibiotic resistance. Future studies should encompass in vivo evaluation, clinical trials, and investigation of synergistic effects with conventional antibiotics to further validate the therapeutic potential of this novel formulation.

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