RESEARCH ARTICLE

Preparation and Evaluation of *Ficus racemosa* Extract Transdermal Gel for Antimicrobial and Anti-inflammatory Activity

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ABSTRACT

Background*: Ficus racemosa* (FR) belongs to the Moraceae family having different pharmacological activities. The primary aim of the researchers was to examine how varying polymer concentrations affect the outcome, additionally nature of penetration enhancers on microbial growth inhibition, and the protein denaturation inhibition capability of *F. racemosa* extract (FRE).

Method: FRE has been prepared by a simple maceration process. By performing an antimicrobial assay procedure and *in-vitro* protein denaturation inhibition method, the antimicrobial and protein denaturation capability of the extract has been confirmed. Furthermore, 12 formulations of transdermal gel were developed with polymer (carbopol 934), which was used at non-identical concentrations (1, 1.5, 2, 2.5%) along with three dissimilar kinds of penetration enhancers, i.e., Tween 80, oleic acid, thioglycolic acid at the same concentration (1%). Transdermal gels have been prepared by a dispersion method & evaluated by physical parameters, viscosity, spreadability, pH, skin irritation, drug content, and *in-vitro* study with Franz diffusion cell.

Result: Formulation B3 showed the best results, having a polymer concentration of 1.5% and Tween 80 as a penetration enhancer.

Keywords: Denaturation, Penetration enhancer, Microbial inhibition, Drug content.

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INTRODUCTION

The phrase transdermal drug delivery system (TDDS) describes dose formulations for individual, direct distribution[1-3] by uniformly dispersing a curative effective dose of the active pharmaceutical agent across the barrier or the patient's skin, these dosage forms achieve their primary goal of releasing an effective agent into the bloodstream at a predetermined rate and amount with a little inter- and intrapatient variation.[4,5] Transdermal administration is now one of the drug administration strategies that shows the most promise.[3,6] It decreases the burden that is frequently placed on the digestive system and liver by other routes, especially oral. It enhances patient compliance, reduces dangerous drug side effects brought on by brief overdoses, and is practical for transdermal drugs that only need a single weak application.[7] That will provide many pros, for instance: appropriate availability of therapeutic agents in bloodstreams, more consistent levels in plasma, and; 3. A prolonged duration of action, which will lead to a decrease in dose frequency, side effects are less, and better therapy because of maintained plasma levels.[8] Delivery via the dermal route enables a consistent supply of medications with quick elimination rate, removing intermittent entry into the systemic circulation and provides controlled, constant drug administration. Despite the wide Delivery via the dermal route enables a consistent supply of medications with quick elimination rate, removing intermittent entry into the blood circulation and provides controlled, constant drug administration.array of transdermal preparations designed to address various topical and internal bodily issues, such as buccal mucoadhesive patches for herpes simplex, transdermal patches for shingles caused by herpes zoster, and transdermal karaya gum patches for warts, along with innovative solutions like microneedles containing trimeric influenza hemagglutinin protein for influenza and dissolving microneedle patches for measles and Oropharyngeal swabs incorporating microneedles and integrated virus-specific antibodies for COVID-19. transdermal gels remain a significant aspect of superficial medication.[9] Gels are frequently utilized as topical dosage forms and lubricants. Gels are usually made from a liquid phase that has been dispersed with other ingredients. Topical gel are intended to the skin or specific

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mucosal surfaces for their particular action or percutaneous penetration of therapeutic agents for their protective action or emollient activity.[10] The release from gel formulations should be similar to that of a simple solution since molecules can freely diffuse through the scaffold of the polymers in the continuous liquid phase. According to an article written by Alberti *et. al*,[11] transdermal gels are also designed for sustained release rate, resulting in systemically consistent levels. Gels are typically considered to be stiffer than jellies because they are more crosslinked covalently and have physical bonds of higher density or are slightly more solid than jellies. However, when compared with patches, gels are often perceived as more advanced due to their greater dosage flexibility, reduced irritation, and improved cosmetic appearance. Moreover, Gel systems can be as transparent as aqua or murky depending on whether the constituents are molecularly dispersed (soluble or insoluble) or if they agglomerate and scatter light. With a few exceptions, the gelling agent's amount is typically less than 10%, typically in the 0.5% to 2.0% range.[12]

However, due to the particularly organized constitution of the skin, which serves as a primary obstacle to drug entry via the skin, this route presents difficulties but these barriers can be circumvented by making use of multifarious other approaches, such as the addition of agents that enhance penetration, other electronic methods like electroporation, iontophoresis, and various nanotechnologically produced nanocarrier systems.[13-15] The most recent one uses nanoparticles, liposomes, dendrimers, ethosome, carbon nanotubes, and many more techniques to get beyond the drawbacks of traditional formulations.[16-18] These techniques support formulation fraternity and another one is stable nanocarriers as an alternative approach to bring drugs on the other side of the skin barrier. Many transdermal medicines, are offered on the market with a unique approach to increase medication penetration for instance including Estrasorb, Diractin, VivaGel, Daytrana are included, Aczone, and Sileryst,. In this research, we have used variant penetration enhancers to augment the penetration rate. These chemical agents work through three main mechanisms[19-21] Firstly, they disrupt the complex structure of stratum corneum lipid, secondly, by improving the partition of the drug in aqueous and non-aqueous media, and by addition of co-enhancer or solvent into the stratum corneum. Furthermore, by causing interaction with intercellular protein.

Penetration through the skin also depends on the few physical and chemical characteristics of a molecule that would penetrate through the stratum corneum well such as oil and water solubility, melting point, its molecular mass. Sulphoxides and similar chemicals such as dimethyl sulphoxides, (1-dodecylazacycloheptan-2-one or laurocapran) pyrrolidones, urea (myristic acid, lauric acid, and capric acid), alcohols, fatty alcohols and glycols, surfactants essential oil, terpenes, and terpenoids cyclodextrins, oxazolidinones[22-25] are used as an enhancer for penetration.

In this research, we have used oleic acid, thioglycolic acid, and tween 80% in varying concentrations to elevate the rate of the drug molecule's penetration through cell membrane. A variety of transdermal gels are available in the pharmaceutical market in which API, polymer, penetration enhancer, and other required excipients are well formulated. Furthermore, researcher frequently uses herbal drugs, additionally their extracts to prepare topical formulations for the treatment of inflammation and pain.[26,27] We have selected FRE for research as it has the capability to inhibit the growth of microbes as well as able to denature the proteins that show their anti-inflammatory activity. FR belongs to the Moraceae family, its class name is Magnoliopsida, and the order is Rosales of Kingdom Plantae. However, while this tree is distributed throughout India, it is particularly abundant in West Bengal. After reviewing of literature, we found that each part of FR possesses therapeutic activity such as hepatoprotective, chemopreventive, hypoglycemic, anti-inflammatory, antipyretic, anti-diuretic anti-tussive, anti-bacterial and antioxidant activities, additionally it has been used in management of dysentery inflammatory conditions, jaundice, biliary disorders, diabetes, and diarrhea according to ayurveda.[28-30]

MATERIALS AND METHODS

FRE has been prepared in the pharmacognosy laboratory at Swami Devi Dyal Institute of Pharmacy. Carbopol 934, propylene glycol, oleic acid, tween 80, triethanolamine, potassium dihydrogen phosphate, disodium hydrogen phosphate, and thioglycolic acid have been procured from Nice Chemicals Ltd.

Collection and Extraction

FR stem bark was harvested from the park located within the Shahabad Co-operative sugar factory in Shahabad Markanda, Kurukshetra, Haryana, which is the species' natural habitat. To lessen the buildup of contaminants, the bark samples of FR were first gently cleaned under running tap water and then briefly treated with water that must be distilled. For 7 to 10 days, the stem bark of FR was sun-dried for 4 to 5 hours each day. Dried stem bark was ground into a fine powder. About 200 gm of powdered drug was dissolved in two-thirds of the amount of ethanol (99.5%) in the ethanolic extraction procedure. The mixture was then thoroughly mixed and refluxed for three to four days, three hours each day. Using the Whatman No. 1 filter, paper extracts were filtered and the filtrates were then evaporated. Room temperature was used to preserve the extracts until further processing.[31]

Antimicrobial Activity

The bacteria used for testing were *Salmonella typhi*, *E. coli*, *K. pneumoniae*, *Candida albicans*, and *Streptococcus aureus*. A revision was made to the agar well diffusion method. Bacterial colonies were maintained on a nutrient agar medium.

The bacterium was added to the culture media in a separate suspension within the nutritional broth. Punching 8 mm diameter wells into the agar, plant extracts (1 mg/mL) and solvent blank (hydro-alcohol) were added. Chloramphenicol, a standard antibiotic, was simultaneously utilized as a positive control at a concentration of 1-mg/mL. This served

as a benchmark to ensure the reliability of the experiment's conditions. After that, the bacterial plates were cultured for 24 hours at 37°C. By measuring the diameter of the observed inhibition zone, the antibacterial activity was assessed. repeated steps are used to ensure antifungal action with Ketoconazole as positive control.[32,33]

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test} * 100}{\text{Absorbance of control}}$$

In-vitro anti-inflammatory activity

The inhibition of the protein denaturation process method has been employed for the *in-vitro* anti-inflammatory activity given as under. For this method, ibuprofen was used as a reference drug.

Preparation of reference drug (positive control)

A fine powder of ibuprofen was made using mortar and pestle. A digital analytical balance was used to weigh the powder and 0.2 g of powdered ibuprofen medication was added to distilled water (20.0 mL). The reference drug, ibuprofen, was successively weakened from 1000 to 0.01 µg/mL then the solutions were combined using a vortex.

Preparation of reaction mixture

About 2.8 mL of phosphate-buffered saline (pH 6.4) and 0.2 mL of fresh hen's egg albumin were mixed to make a final volume of 5 mL. Then, reaction mixtures were gently mixed with 2 mL of the ethanolic extract of FR bark from each distinct concentration. Ibuprofen was one of the reference medications that underwent a similar process and served as the study's positive control. Furthermore, reaction mixtures have been incubated for 15 to 20 minutes at 37 ± 2°C in a water bath to inhibit denaturation of proteins. The reaction mixture is then heated to 70°C and kept there for 5 minutes. After that, the reaction mixture was given 15 minutes to cool at room temperature. Using a colorimeter, the absorbance of the reaction mixture was measured at 680 nm for each concentration (1000, 100, 10, 1, 0.1, and 0.01 µg/mL) before and after denaturation.[34,35] The mean absorbance was measured after each test was conducted three times and calculation for %protein denaturation inhibition was done using the following equation.

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test} *}{\text{100Absorbance of control}}$$

Preformulation Studies

Drug-excipient interaction study

The samples were examined using FTIR spectroscopy as part of extract-excipient compatibility research to look at potential interactions between FRE and other excipients, such as carbopol 934.[36,37]

Preparation of calibration curve of β-sitosterol

A 10 mL standard flask containing 10 mg of β-sitosterol was filled with phosphate buffer (pH 6.8). Using phosphate buffer (pH 6.8), the stock solution's volume was increased to 10 mL. Aliquots of 0.5, 1, 1.5, 2, 3, 4, and 5 mL were poured from the aforesaid stock solution to a 10 mL volumetric flask. By using phosphate buffer pH 6.8 the volume was adjusted, yielding a final standard concentration of 50, 100, 150, 200, 300, 400, and 500 µg/mL. By measuring the absorbance of secondary stock solutions for β-sitosterol at 208 nm in a UV double-beam spectrophotometer, the standard curve was plotted.[38-40]

Formulation Studies

Preparation of FRE transdermal gel

Three penetration enhancers have been used with varying concentrations. By using the formula given in Table 1 transdermal gels have been prepared by dispersion method**.** The necessary amount of Carbopol 934 has been combined in a beaker with slightly warm water. The measured amount of propylene glycol and ethanolic FRE were added to a different beaker and thoroughly mixed. Next, the drug-propylene glycol mixture was introduced gradually using a glass rod. After that, penetration enhancers were added dropwise with stirring. Stirring was continued with dropwise addition of triethanolamine till a smooth, transparent gel was formed.[41]

Evaluation Parameters of the Prepared Transdermal Gels[9,26,41,42].

Appearance

Preparations were visually inspected for colour.

pH determination

The pH was determined by dispersing 1 g of gel in distilled water quantity was 100 mL, allowing it to sit for two hours. The pH was then measured and documented three times using a digital pH meter on different occasions**.**

Viscosity study

The Brookfield viscometer was used to record the viscosities of several FRE gels that were made at 250 and 10 rpm.

Spreadability

The wooden block slide apparatuses have been used to test the spreadability of various transdermal gels. The amount of duration in seconds required to separate the slides was recorded after applying weight. Each formulation's spreadability was quickly determined. Furthermore, dissemination of value is essential for therapeutic efficacy. The spreadability is computed using the formula below.

Spreadability (S) = M × L / T

Where M is the weight that tied to the upper slide, L is the glass slides's length and T is the amount of time taken to separate the slides

Skin irritation study

There were three volunteers involved. Two inches square of the volunteer's hand skin was treated with one gram of FRE transdermal gel. The skin was visually examined and various parameters were assessed, including erythema (redness), edema (swelling), and other signs of irritation such as itching or burning sensation.

Table 1: Composition of FRE gel formulations (% w/v)

Ingredients	*A1*	*A2*	*A3*	*A4*	*B1*	*B2*	*B3*	*B4*	*C1*	*C2*	*C3*	*C4*
FRE Ethanolic extract (mg)	300	300	300	300	300	300	300	300	300	300	300	300
Carbopol934	1	1.2	1.5	2.0	1	1.2	1.5	2.0	1	1.2	1.5	2.0
Oleic acid	1	1	1	1	-	-	-	-	-	-	-	-
Tween 80	-	-	-	-	1	1	1	1	-	-	-	
Thioglycolic acid	-	-	-		-	-	-		1	1	1	1
Propylene glycol	10	10	10	10	10	10	10	10	10	10	10	10
Triethanolamine	5	5	5	5	5	5	5	5	5	5	5	5
Distilled water (q.s)	100	100	100	100	100	100	100	100	100	100	100	100

In-vitro cellophane membrane permeability studies

For *in-vitro* permeation studies, modified Franz diffusion cells were utilized. The volume of the receiver compartment was 60 mL, and the effective diffusional area was 3.56 cm^2. A cellophane membrane was mounted between the two compartments of the diffusion cell. Subsequently, gel weighing accurately 1 g, containing 1-mg of FRE, was poured into the donor cell. To maintain sink conditions, phosphate buffer pH 7.4 was added to the receiver cell. Furthermore, the effective diffusional area remained at 3.56 cm^2. The cell was then placed on a magnetic stirrer, with the temperature maintained at 37 ± 0.5°C. The content was stirred with the assistance of a magnetic bar in the receiver compartment. At predetermined intervals (0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hours), 2 mL permeate samples were replaced with an equal amount of drug-free solvent (PB pH 7.4) from the receiver compartment. Moreover, collected permeate samples were evaluated for β-sitosterol content using the UV spectroscopic method at 208 nm.

Drug content

After precisely weighing and dissolving 1.0 g of the produced gel in 100 mL of distilled water, the mixture was sonicated for three hours using a bath sonicator and filtered through a 0.45 μm membrane filter. Additionally, the filtrate was appropriately diluted and analyzed using a UV spectrophotometer.

RESULTS

Antimicrobial Activity

FRE showed a significant inhibitory effect against *Streptococcus aureus, Salmonella typhi*, *K. pneumoniae*, *Candida albicans, and E. coli.* MIC values are: 1.89, 1.56,1, 57, 0.78, 1.56 for tested microbes, respectively. The results are displayed in Table 2.

Anti-inflammatory Activity

Protein denaturation inhibition anti-inflammatory activity was accessed. Results are given in Table 2 for the anti-inflammatory activity of FRE in comparison to ibuprofen as a reference drug. The FRE at 20 mg/100 gm body weight (B.W) showed 32.52% inhibition of denaturation while 68.32% at 40 mg/100 gm B.W. As the inhibition of denaturation of ibuprofen at 40 mg/100 gm B.W was 69.52% the studies revealed that FRE has a satisfactory anti-inflammatory activity.

Preformulation studies

Drug excipient interaction studies

The FTIR data showed no interaction between FRE and the excipients, as the presence of excipients did not alter the distinctive peaks of the extract (Figure 1).

Calibration curve of β-sitosterol

The standard curve of FRE was prepared in phosphate buffer (pH 7.4), and the absorbance data obtained underwent linear regression. The correlation coefficient was found to be 0.9997, and the standard curve is shown in Figure 2. The derived equation represents a straight line, expressed as $Y = 0.0935x + 0.0002$.

Evaluation Parameters of the Prepared Transdermal Gel Formulations

All prepared formulations underwent to multiple evaluation parameters. It was found that all the formulations were Brownish-yellow in appearance the drug content was found to be within the limits as written in the literature. All formulations showed a neutral pH value between 7.2 to 7.4, which is required for application on skin. The viscosity became directly proportional to the concentration of polymer and varied from 323.6 to 3748 cps. The spreadability of the formulation varied from 15.3 to 53 seconds. The formulations had sufficient spreadability for spreading on the skin. All the formulations showed no skin irritation when applied topically. The data is tabulated in Table 3.

In-vitro release studies

In-vitro release experiments were performed on all formulations to evaluate how polymer concentration and penetration enhancers influence the release rate over an 8-hour duration. The formulations A1 to A 4 had varied amounts of Carbopol 934 from 1 to 2% and oleic acid was used as a penetration enhancer. A1 showed a maximum release of 38.70 ± 042% at 8 hours as the concentration of polymer was increased from 1 to 1.5% the release rate increased to 61.78 ± 0.39 but decreased when the concentration was increased to 2% (55.93 ± 0.18). This could be due to higher entrapment of drug by the polymer matrix. The formulation B1 to B4 contained Tween 80 as a penetration enhancer. B3 in this group

Table 2: Inhibition of protein denaturation

Protein denaturation test. group	*Absorbance at 660 nm*	*Inhibition of denaturation (%)*
Control	1.26 ± 0.006	-
Ibuprofen 20 mg/100 gm B. W.	1.02 ± 0.007**	39.05
Ibuprofen 40 mg/100 gm B. W	0.61 ± 0.007**	69.52
FRE 20 mg/100 gm B. W.	1.24 ± 0.17**	32.52
FRE 40 mg/100 gm B. W.	0.490 ± 0.005**	68.32

mean ±.S.D.(n = 3), ***p* < 0.01

shown maximum release with value 70.93 ± 0.18. The formulations C1 to C4 were formulated using thioglycolic acid as a penetration enhancer and C3 in this group at 50.51 ± 0.15% was shown maximum result. The formulation B3 containing 1.5% Carbopol 934 and 1% Tween 80 as permeation enhancer is a better formulation which showed the highest drug release in the study of 8 hours as compared with plain drug gel, which showed only 29.21+0.5% of drug release. The drug release data is shown in Figures 3-6.

DISCUSSION

FR has been extensively studied for its remarkable therapeutic properties. These include antioxidant, antidiuretic,

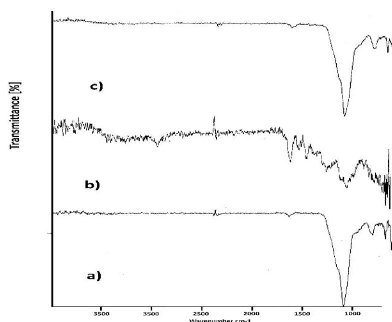

Figure 1: FTIR spectrum of a) Carbopol 934, b) FRE and c) Physical mixture of Carbopol 934and FRE

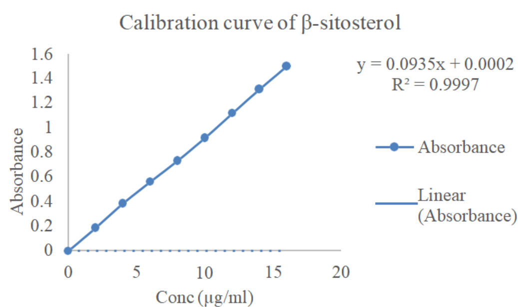

Figure 2: Calibration curve of β-sitosterol

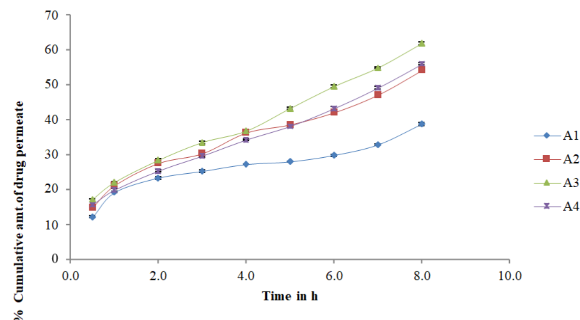

Figure 3: *In-vitro* drug release profiles of formulations A1-A4 (mean ± S.D, n = 3)

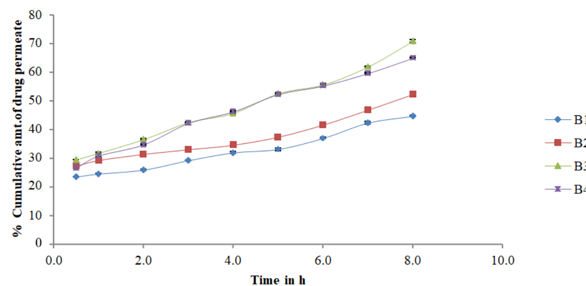

Figure 4: *In-vitro* drug release profiles of formulations B1-B4 (mean ± S.D, n = 3)

Table 3: Evaluation data of FRE gel formulations

Formulation code	*Drug content(% w/v)*	*Appearance*	*pH*	*Viscosity (cps)*	*Spreadability(s)*	*Skin irritation*
A1	100.15 ± 1.753	Brownish-yellow	7.27 ± 0.153	323.6 ± 2.2	15.3 ± 1.24	Nil
A2	100.19 ± 1.289	Brownish-yellow	7.27 ± 0.058	403 ± 3.5	16 ± 1.63	Nil
A3	99.2 ± 1.136	Brownish-yellow	7.27 ± 0.153	448.3 ± 4.3	15 ± 1.63	Nil
A4	100.46 ± 0.977	Brownish-yellow	7.17 ± 0.115	529.3 ± 4.9	19 ± 1.63	Nil
B1	100.54 ± 0.836	Brownish-yellow	7.27 ± 0.115	1423 ± 3.7	30 ± 0.28	Nil
B2	100.47 ± 1.61	Brownish-yellow	7.13 ± 0.058	1426 ± 5.3	30 ± 1.69	Nil
B3	100.35 ± 1.197	Brownish-yellow	7.23 ± 0.153	1525 ± 2.4	30 ± 2.16	Nil
B4	99.88 ± 1.361	Brownish-yellow	7.23 ± 0.153	1635.6 ± 3.2	35 ± 2.44	Nil
C1	100 ± 1.163	Brownish-yellow	7.3 ± 0.173	2526 ± 3.74	41 ± 1.24	Nil
C2	100.66 ± 1.194	Brownish-yellow	7.37 ± 0.115	2536 ± 3.39	45 ± 1.24	Nil
C3	100.44 ± 1.143	Brownish-yellow	7.33 ± 0.208	2674 ± 3.74	47 ± 2.05	Nil
C4	99.93 ± 1.571	Brownish-yellow	7.23 ± 0.153	3748 ± 2.44	53 ± 2.05	Nil

mean ±.S.D.(n = 3)

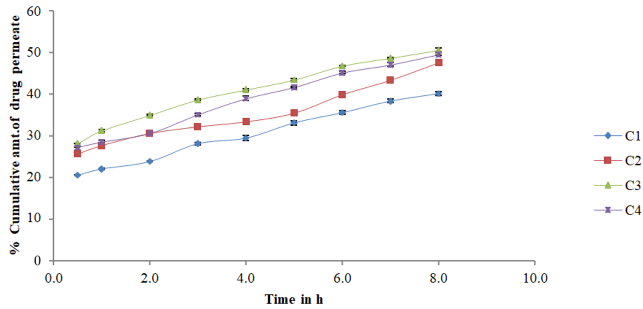

Figure 5: *In-vitro* drug release profiles of formulations C1-C4 (mean ± S.D, n = 3)

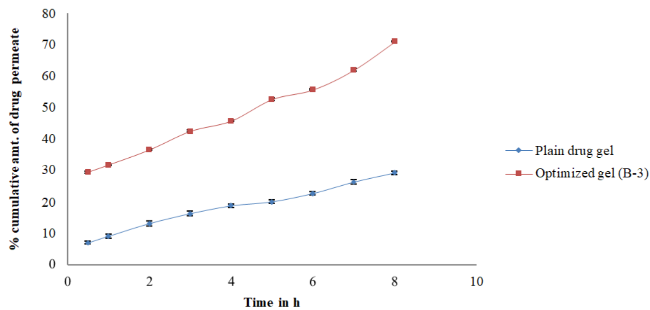

Figure 6: *In-vitro* drug release profiles of formulations B3 and Plain Gel (mean ± S.D, n = 3)

hypolipidemic, hepatoprotective, analgesic, antipyretic, hypoglycemic, antimicrobial, and anti-inflammatory activities. Its diverse range of beneficial effects can be attributed to several active constituents, including flavonoids, diterpenoids, rographolides, β- sitosterol, stigmasterols, campsterol, and α-amyrin. The study showed that FRE has significant antimicrobial and anti-inflammatory activity.

Additionally, surfactant Tween 80 plays a crucial role in enhancing drug delivery. By reducing interfacial tension, it increases contact area and permeability. The formulation labeled B3 has been validated through various tests, affirming its efficacy. Furthermore, the penetration rate is influenced not only by the nature of the penetration enhancer but also by the concentration of the polymer.

In summary, FR holds immense promise as a therapeutic agent, and its multifaceted benefits continue to be explored in research endeavors.

CONCLUSION

The transdermal gel was formulated using the dispersion method. Our investigation focused on assessing the impact of two key formulation variables: the quantity of polymer and the choice of penetration enhancer. Our findings indicate that formulation B3, which includes 1% Tween 80 as a penetration enhancer and 1.5% Carbopol 934 as the polymer, yielded the most promising results. This conclusion was drawn based on experimentation conducted using a cellophane membrane embedded within a Franz diffusion cell. Further validation of these results could involve *in-vivo* studies conducted on animals. Such studies would offer deeper insights into the gel's efficacy, safety, and potential for therapeutic application.

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