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Development and Evalution of Gel formulation for *Holoptelea Integrifolia* Planch. Extract Loaded Solid Lipid Nanoparticles

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Abstract

The therapeutic potential of Holoptelea integrifolia Planch., a medicinal plant widely used in traditional medicine, has gained significant attention due to its diverse pharmacological activities. However, the effective delivery of its bioactive compounds remains a challenge due to their poor solubility, low bioavailability, and instability. In this study, we aimed to develop and evaluate a gel formulation loaded with Holoptelea integrifolia Planch. extract-loaded solid lipid nanoparticles (SLNs). The gel formulation was evaluated for rheological properties, stability, and in vitro release kinetics. The results exhibited that the developed gel formulation exhibited desirable rheological characteristics, providing ease of application and prolonged contact time with the skin. The gel formulation demonstrated sustained release of the bioactive compounds, ensuring controlled and targeted delivery. The findings of this study highlight the potential of the gel formulation as a promising topical delivery system for Holoptelea integrifolia Planch. extract, offering enhanced therapeutic efficacy for various dermatological conditions.

Keywords: Holoptelea integrifolia, SLNs, Gel Formulation, In vivo, Anti-fungal activity

1. INTRODUCTION

In recent years, a growing interest in natural products for their potential therapeutic properties and minimal side effects has been observed (Tsivileva et al., 2022). Among these, Holoptelea integrifolia Planch., commonly known as the Indian elm or Chilbil, has gained considerable attention due to its diverse medicinal properties (Kumar et al., 2012). It has been traditionally used for the treatment of various ailments, including skin disorders, inflammation, and microbial infections (Somwong et al., 2022).

Holoptelea integrifolia Planch. extract is rich in bioactive compounds such as flavonoids, tannins, saponins, and phenolic compounds, which contribute to its pharmacological

activities. However, the delivery of these bioactive compounds to the target site remains a challenge due to their poor solubility, low bioavailability, and instability (Subramanyam et al., 2017). To overcome these limitations, novel drug delivery systems such as solid lipid nanoparticles (SLNs) have gained significant attention in recent years (Nunes et al., 2017).

SLNs offer several advantages for the encapsulation and delivery of bioactive compounds, including enhanced stability, controlled release, improved bioavailability, and the potential to target specific sites. Furthermore, incorporating SLNs into a gel formulation provides the added advantage of ease of application, prolonged contact time with the skin, and improved patient compliance.

In this research study, our objective was to develop and evaluate a gel formulation loaded with Holoptelea integrifolia Planch. extract-loaded solid lipid nanoparticles. The encapsulation of the extract within SLNs was achieved using a biocompatible lipid matrix, enabling the protection and controlled release of the bioactive compounds. The developed gel formulation aimed to provide enhanced therapeutic efficacy for various dermatological conditions, taking advantage of the synergistic effects of the bioactive constituents present in Holoptelea integrifolia Planch. Extract (Mukherjee et al., 2009; Alsaad et al.,2020; Trombino et al.,2016).

The research work involved a systematic approach, including the preparation of plant extract and evaluation of the gel formulation of plant extract loaded SLNs for rheological properties, stability, and in vitro release kinetics (Nagaich et al., 2016; Malik et al., 2018).

The findings of this study are expected to contribute to the development of a novel and effective topical formulation for Holoptelea integrifolia Planch. extract, which can potentially be utilized as an alternative therapeutic option for various skin disorders. The optimized gel formulation could provide improved drug delivery, enhanced skin penetration, and targeted release of the bioactive compounds, thereby maximizing their therapeutic benefits.

Overall, this research aims to bridge the gap between traditional knowledge and modern pharmaceutical technology by developing a formulation that harnesses the potential of Holoptelea integrifolia Planch. extract for dermatological applications, while also addressing the limitations associated with its natural form.

2. MATERIAL METHODS

2.1 Preparation of Ethanolic Extract of *Holoptelea integrifolia* Leaves

The Soxhletation method was used for the effective extraction of active constituents from *Holoptelea integrifolia* leaves. It allows for the continuous extraction of the plant material

and yields a high-quality extract. The leaves were firstly collected and dried at room temperature to remove moisture content. The dried leaves were then ground into a fine powder using a mortar and pestle and packed the powdered leaves into a Soxhlet thimble which made up of glass wool. The thimble is placed in the Soxhlet apparatus, and the flask is filled with ethanol as a suitable solvent. The extraction process involved repeated cycles of solvent circulation through the thimble containing the plant material. The extraction process continued for 8 hours until the active constituents were completely extracted from the plant material (Kumar et al., 2019). The extracted solution was then filtered, concentrated, and dried using a rotary evaporator to obtain the crude extract.

2.2 Peparation of Solid Lipid Nanoparticles Laoded with Leaf Extract

The preparation of *Holoptelea integrifolia* ethanolic leaf extract loaded solid lipid nanoparticles were prepared through a ultrasonication method. These prepared SLNs also charcterized to understand theri impact on efficacy and safety in pharmaceutical and biomedical applications.

2.3 Development of Hydrophilic Gel Formulation

The optimized formulation has highest EE, optimum vesicle size and ZP which was further utilized to incorporated into Carboxyvinyl polymer carbomer (Carbopol 934P) for the gel formulations. Carbopol 934P (1.0% w/w) was soaked in water for an hour and add 10 ml of nano-vesicular dispersion containing *Holoptelea integrifolia* leaf extract (10 mg). Stirred the mixture at 800 rpm at 30ºC to make homogeneous gel. Maintain the neutral pH of prepared gel by triethanolamine (Rarokar et al., 2022).

2.4 Characterization of Hydrophilic Gel Formulation

2.4.1 Viscosity and pH measurement

The viscosity of SLNs containing gel formulation was measured using Brookfield viscometer (Model No DV-III ULTRA) with spindle no 06 at 100 rpm and pH measurements of the formulations were done using digital pH meter (RI-152-R).

2.4.2 Spreading Diameter

The spread ability of SLNs containing gel formulation was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm x 20 cm) after 1 min. The standard weight applied on the upper plate was 125 gm.

2.4.3 In vitro release studies

In vitro release study of gel formulation was determined and compared with a conventional gel formulation using dialysis membrane (Hi-media). The gel was placed in PBS (7.4) for 4 hrs and then mounted between the donor and receptor compartment of the Franz diffusion cell (surface area for diffusion was 2.54 cm^2). To perform ex vivo permeation study using animal skin full-thickness goat ear skin (procured from local abattoir; used within 24 h) was used. The skin was mounted in between receptor and donor compartments with the stratum corneum side facing upward into the donor compartment. The release rate of *N. sativa* was analyzed by placing the required sample in the donor cell compartment. To prevent contamination and evaporation, the donor compartment was covered with parafilm. The receptor chamber was filled with phosphate buffer PBS (7.4) and was maintained at 37ºC with continuous stirring. 1 ml aliquot of receptor phase solution was withdrawn at time intervals of 0.5, 1, 2, 4, 8 and 24 hr and the same volume of fresh medium was added back into the chamber. The quantification was done using UV spectrophotometer (Shimadzu Model No. 1800) at 203 nm. The cumulative amount of drug permeated per unit area versus time graph was plotted and transdermal flux (J) was calculated from the slope of linear portion.

2.4.4 Permeation data analysis

To study the release rate profile the data obtained from in vitro drug release study were fitted in different kinetic equations: zero order as the cumulative percent of drug remaining vs. time, first order as the log cumulative percentage of drug remaining vs. Time, Higuchi's model as the cumulative percent drug remaining vs. square root of time, Hixson Crowell cube root model and Korsmeyer- Peppas model as the log cumulative percentage of drug released vs. log time.

2.4.5 Drug content of the formed gels

500 mg of the gel was taken and dissolved in 50 ml of pH 7.4 phosphate buffer (PBS). The solution was then passed through the filter paper and 50 µl of the filtrate was withdrawn. The filtrate was diluted by adding 3.5ml of distilled water and the drug content was measured spectrophotometrically at 203 nm against corresponding gel concentration.

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2.4.6 In vivo anti-fungal activity

In vivo antifungal activity was performed for the optimized formulation as per OECD guideline (Dzoyem et al., 2014; Jia et al., 2018). The animal experimentation was performed according to institutional ethics committee granted permission under wide letter (Letter No. **1196/PO/Re/S/08/CPCSEA**).

Experimental animals for antifungal activity

The pathogen-free albino Wistar mice with no previous drug treatment were selected for *in vivo* antifungal studies. Healthy albino mice (6–8 weeks old) were received from the animal house of Truba Institute of Pharmacy, Bhopal (M.P.) India, placed in polypropylene cages with controlled temperature (25 °C), humidity and 12 h light/dark cycles, and fed on a standard diet and water *ad libitum.* Acclimatization to laboratory hygienic condition was done for seven days before starting the experiment.

Induction of Fungal Infection

Intravenous (i.v.) injection of 0.2 mL of a 106 UFC/mL inoculum formed in sterile saline using a fresh 48 h Candida albicans culture resulted in disseminated candidiasis infection in rats. Three animals were sacrificed after 24 hours of the infection to evaluate the efficacy of infection by measuring the fungus load in the blood.

Treatment of Fungal Infection

Infected rats were housed in cages with unrestricted access to food and water and were divided into six groups of 6 animals each. 0.5, 1 and 2 g/kg of body weight of leaf extract gel was given topically over three days starting 24 hours after infection. A positive control group was administered with a reference antifungal drug Fluconazole at dosage of 10 mg/kg of body weight, whereas the untreated control got distilled water. The mortality of mice was monitored daily for 15 days after fungal inoculation.

Table 1: Groups and treatment for the in vivo anti-fungal activity

Group Number	**Treatment**
Group 1	Untreated
Group 2	Control (Distilled water)
Group 3	Positive Control (Fluconazole 10 mg/ Kg bw)
Group 4	0.5 g/kg of body weight of *Holoptelea integrifolia leaf* extract gel

Group 5	1 g/kg of body weight of *Holoptelea integrifolia leaf* extract gel
Group 6	2 g/kg of body weight of *Holoptelea integrifolia leaf* extract gel

Animal Used: Albino rats (6 in each group); Condition: Controlled Temperature (25 ±2 ^{o} C), Relative Humidity (60 ± 5 %)

Statistical Analysis

The significance differences between the experimental group and control group were analysed with the help of one-way ANOVA using Student-Newman-Keuls test. A value of $P < 0.05$ at a 5% level of significance was considered to be significant. All data were analysed using SPSS Statistics 17.0.2.

3. RESULT AND DISCUSSION

3.1 Development and Characterization of Hydrophilic Gel Formulation

Tthe carboxyvinyl polymer carbomer (Carbopol 934P) was used to incorporate with optimized solid lipid nanoparticles for the preparation of gel formulation.The various parameters were observed for the characterization of hydrophilic gel formulation.

3.1.1 Viscosity, pH and spreading diameter measurement of gel formulation

The prepared SLNs containing gel was characterized with the help of different properties like viscosity, pH, spreading diameter and drug content. All these characteristics properties were suitable as per the topical application and mentioned in **Table 2**.

Table 2: Evaluation of physicochemical properties of *Holoptelea integrifolia* leaf extract loaded SLNs gel formulation

Formulation Code	**Colour**	**Homogeneity**	**Texture**	**Viscosity (centipoise)**	**pH**	**Spreading diameter after 1 min (mm)**	**Drug content (%)**
SLN2-GEL	White	Homogenous	Smooth	4073±5.7	6.4±0.5	54 ±3.2	75.1±2.1

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3.1.2 *In vitro* release of *H. integrifolia* leaf extract loaded SLNs and its gel formulation through membrane

In vitro drug release through dialysis membrane showed 25.6%, 31.7 % and 13.4% extract released from SLN2, SLN2-GEL and Ethanolic extract solution (EX) respectively in 4 hrs, while marketed formulation (Daktarin Gel, Janssen Pharmaceuticals Pvt Ltd) showed 11.2% drug release. Within 24 hrs total amount of drug release was found to be 84.2%, 97.3%, 61.2% and 53.3% respectively from SLN2, SLN2-GEL, EX and marketed gel.

Table 3: Percent cumulative drug release after 24 hrs from various SLNs containing gel formulation through membrane

Time	Cumulative % Drug Release			
	SLN2	**SLN2-GEL**	**Extract (Ex)**	**Marketed Gel (Mgel)**
0	0	0	0	0
4	25.6 ± 3.2	31.7 ± 2.7	13.4 ± 3.1	11.2 ± 2.9
8	43.1 ± 2.5	55.3 ± 2.2	24.4 ± 2.6	19.4 ± 3.1
12	59.8 ± 2.7	67.4 ± 3.8	35.6 ± 1.8	28.8 ± 1.6
16	70.7 ± 2.9	82.9 ± 1.7	46.2 ± 3.5	34.6 ± 3.4
18	79.6 ± 3.4	92.1 ± 2.9	53.5 ± 2.7	45.2 ± 2.9
24	84.2 ± 3.6	97.3 ± 2.4	61.2 ± 1.9	53.3 ± 2.8

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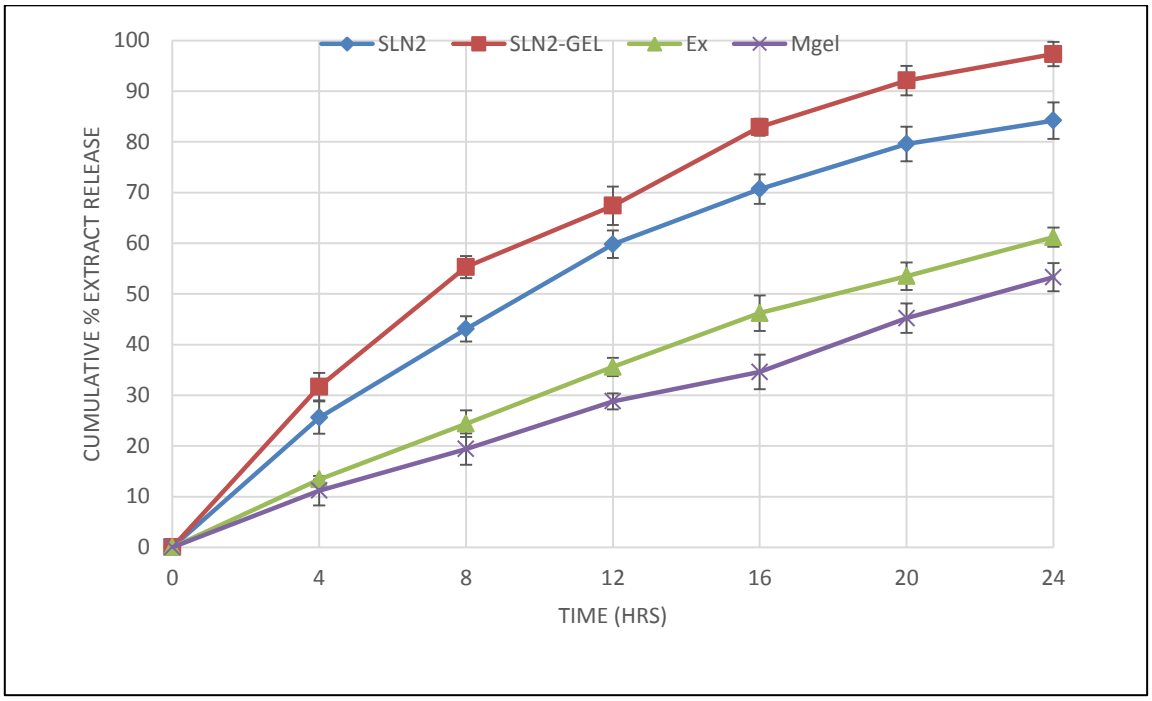

Fig. 1: Percent cumulative drug release after 24 hrs from SLNs containing gel formulation through membrane

3.1.3 *In vitro* release of *H. integrifolia* leaf extract loaded SLNs and its gel formulation through animal skin

In vitro drug release through animal membrane, the total amount of drug release was found to be 78.2%, 85.2%, 61.2% and 51.2% respectively from SLN2, SLN2-GEL, EX and marketed gel within 24 hrs. Cumulative percent extract release (CDR) for both SLN2 and SLN2-GEL was significantly higher than marketed formulation ($p < 0.05$).

Table 4: Percent cumulative drug release after 24 hrs from SLNs containing gel formulation through animal membrane

Time	Cumulative % Drug Release			
	SLN2	SLN2-GEL	Extract (Ex)	Marketed Gel (Mgel)
0	0	0	0	0
4	18.4 ± 2.2	23.4 ± 3.2	10.3 ± 3.1	9.2 ± 1.9
8	39.3 ± 3.5	45.1 ± 4.2	24.4 ± 2.3	19.4 ± 2.1
12	52.6 ± 4.7	56.6 ± 3.9	34.6 ± 2.8	28.8 ± 2.6
16	63.8 ± 3.9	67.9 ± 4.7	46.4 ± 3.2	38.6 ± 2.8

18	72.9 ± 2.9	79.2 ± 4.2	53.5 ± 2.3	45.2 ± 2.4
24	78.2 ± 4.1	85.2 ± 3.4	61.2 ± 2.9	51.2 ± 3.8

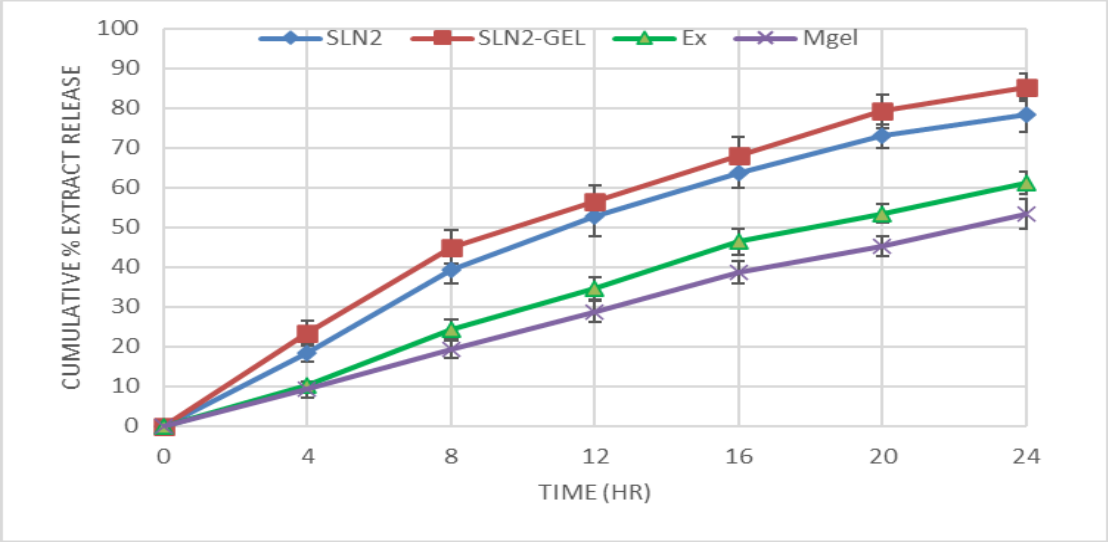

Fig. 2: Percent cumulative extract release after 24 hrs from SLNs containing gel formulation through the animal skin

4. CONCLUSION

In this research, a gel formulation of Holoptelea integrifolia Planch. extract-loaded solid lipid nanoparticles (SLNs) was formulated successfully for topical delivery. The incorporation of SLNs improved stability, controlled release, and enhanced skin penetration. The optimized gel formulation exhibited favorable rheological properties and sustained release of bioactive compounds. It offers potential as an alternative treatment for dermatological conditions, harnessing the medicinal properties of Holoptelea integrifolia Planch. extract. This research highlights the integration of traditional knowledge with modern pharmaceutical technology and provides a promising approach for the development of natural-based formulations for improved therapeutic outcomes. Further studies are needed to evaluate its efficacy and safety in human subjects.

CONFLICT OF INTEREST

The authors declare no conflict of interest financial or otherwise.

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