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FORMULATION DEVELOPMENT, PHYSICOCHEMICAL CHARACTERIZATION AND IN-VIVO WOUND HEALING ACTIVITY OF HIBISCUS ROSA SINENSIS LEAVES EXTRACT LOADED NANO EMULSION

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Abstract

Wounds are tissue damage that can cause functional disorders and risk of infection. Effective wound management is essential to accelerate tissue regeneration and prevent complications. Hibiscus Rosa sinensis leaves extract contains bioactive compounds such as flavonoids, tannins, and saponins that have anti-inflammatory, antibacterial, and wound healing activities. However, the limited bioavailability and skin penetration of these active compounds are challenges in topical therapy. This study aims to develop and evaluate a topical drug delivery system based on Hibiscus Rosa sinensis leaves extract nanoemulsion as an alternative therapy to accelerate wound healing. Extraction was carried out using ethanol solvent, then formulated into a nanoemulsion with oil, distilled water, surfactant and co-surfactant components. Formulation characterization was carried out by measuring particle size, polydispersity index, zeta potential, and physical stability tests. Evaluation of effectiveness was carried out using a wound model in Sprague Dawley rats with the hot plate induction method. Wound healing observations were carried out clinically and histologically for a period of 15 days. The formulation results showed that the nanoemulsion had a particle size of <200 nm, PDI <0.5, and zeta potential of ±25 mV, indicating good physical stability. Wound activity tests showed that topical administration of Hibiscus Rosa sinensis leaves extract nanoemulsion accelerated wound contraction, increased epithelialization, and improved tissue structure compared to the control group. Hibiscus Rosa sinensis leaves extract can be formulated in a nanoemulsion delivery system that shows stable physical characteristics and is effective in accelerating wound healing.

1. Introduction:

In topical dispersion, skin being a primary defense barrier, regards the API's as foreign components and prevents their admission into the body (Phatale et al;2022). The first and roughest layer to penetrate for drug penetration into the skin is the stratum corneum, the outermost layer of the epidermis (Szumała, Macierzanka, 2022). Various mechanisms have been studied to enhance the medication penetration. Disruption of the structure of the skin layer is one such mechanism, which can be produced by means of methods including chemical penetration enhancers, ultrasound, iontophoresis, sonophoresis, electroporation, and microneedles (Gupta et al; 2019).

Nano-emulsions are a new type of emulsion which can be defined as an emulsion with uniform and extremely small droplet sizes, typically in the range of 20–200 nm. Due to their small droplet size, it looks like transparent or translucent. It is also kinetically stable against sedimentation or creaming for a long period of time because of their small droplet size. The use of nanoemulsions in oral dosage forms achieve promising results in higher targeting efficacy of drug, and also increases the drug bioavailability, enhanced permeability and therapeutic functions. [1]

The term "Nanoemulsion" refers to a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules. [2] A nanoemulsion is considered to be a thermodynamically or kinetically stable liquid dispersion of an oil phase and a water phase, in combination with a surfactant. Nano-emulsions are a new type of emulsion which can be defined as an emulsion with uniform and extremely small droplet sizes, typically in the range of 20–200 nm. Due to their small droplet size, it looks like transparent or translucent. It is also kinetically stable against sedimentation or creaming for a long period of time because of their small droplet size. The use of nanoemulsions in oral dosage forms achieve promising results in higher targeting efficacy of drug, and also increases the drug bioavailability, enhanced permeability and therapeutic functions. [1]

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Because nanoemulsions have small droplets, active ingredients can be uniformly deposited and penetrated through the skin's surface (Sonnevilleaubrun, 2004).Due to their wide surface area and low surface tension (Bouchemal et al; 2004), nanoemulsions have better penetration efficacy of the components and require only 3–10% surfactants during manufacture (Tan et al; 2016).

An important aspect to take into account when creating nanoemulsions is the effect of the order in which the various compounds are combined during formulation (Preeti et al; 2023). This makes it essential to stress that in order to develop nanoemulsions, surfactants must be combined with the oily phase first, resulting in conditions that are highly favorable for their formation; on the other hand, combining surfactants with water at the beginning of the preparation process would favor the creation of "macroscopic" emulsions (Dehghani et al; 2017).The many techniques used to prepare nanoemulsions to date can be broadly classified as either low-energy or high-energy emulsification approaches, or a combination of the two (Maruno et al; 2009). High-energy procedures are defined by the

application of mechanical devices that provide powerful disruptive forces which break up the oil and water phases to form the oil droplets. Such a methodology uses high-pressure homogenizers, microfluidizers and sonication methods (Anton et al; 2011).Low-energy methods, on the other hand, emulsify using the system's inherent chemical energy (Ngan et al; 2014).This gets done by rerouting the formulation's inherent physicochemical characteristics of the excipients, co-surfactants, and surfactant (Anton et al; 2008).

Originating in China, the Hibiscus rosa sinensis L. (Malvaceae) herb is renowned for its beautiful, captivating blooms (Sharma & Sharma, 2023). This shrub is widely cultivated in tropical regions as an ornamental plant, presenting various forms with flowers of different colors (Akpan, 2007). Beyond its aesthetic appeal, Hibiscus also possesses medicinal properties, making it a key ingredient in numerous herbal teas (Dahanukar et al;2000).

Because of its therapeutic uses, the red-flowered variety is especially preferred among the others.

(Sharma & Sharma, 2023). Numerous studies have reported that different varieties of Hibiscus plants exhibit varying medicinal properties (Riaz & Chopra, 2018). However, this review primarily centers on exploring the therapeutic potential of the Hibiscus rosa sinensis plant and its wide-ranging applications in traditional and modern medicine (Sharma et al;2023). Some of the major chemical constituents of Hibiscus rosa sinensis are Cholesterol, Compestrol, Stigmasterol, Glucose, Fructose, Flavanoids, Hibiscetin, Cyanin, Glycosides, Alkanes, etc (Jan, 2008).

The roots of Hibiscus rosa sinensis Linn have the ability to suppress coughs. You can treat scalp diseases, inhibit premature graying, and promote hair development with leaves and blossoms (Khan et al;2017). There are reports that the blossoms are useful in treating leprosy, diabetes, epilepsy, and heart issues. The root's infusion can be used to treat venereal diseases (Sivaraman et al;2021). Use the leaves and roots to stimulate blood flow, which aids in controlling menstruation. Utilize the leaves as a means of stimulating placenta ejection following childbirth and as an abortifacient (Bala et al ;2022). Hibiscus rosa sinensis blossoms are used to treat a range of conditions, such as liver issues, high blood pressure, and stomachaches. Hibiscus rosa sinensis fruits are used to cure wounds, sprains, and other diseases. The fruits also have diuretic effects (Sargam et al;2013).

Wound healing is an important but complicated process in human or animal, containing a multifaceted process governed by sequential yet overlapping phases, including hemostasis/ inflammation phase, proliferation phase, and remodeling phase. 1 After an injury to skin, the exposed sub-endothelium, collagen and tissue factor will activate platelet aggregation, which results in degranulation and releasing chemotactic factors (chemokines) and growth factors (GFs) to form the clot, and all above-mentioned procedures will achieve

suc- cessful hemostasis. 2 Neutrophils, the first cells to appear at the injury site, cleanse debris and bacteria to provide a good environment for wound healing. In the following, macro- phages accumulate and facilitate phagocytosis of bacteria and damage tissue. 3 The hemostasis and inflammatory phase often takes 72 h to finish. The following proliferative phase is characterized with an accumulation of lots of cells and profuse connective tissue. The wound encompasses fibroblasts, keratinocytes, and endothelial cells. Extracellular matrix (ECM), including pro- teoglycans, hyaluronic acid, collagen, and elastin forms a granulation tissue to replace the original formation of clot. 4 Many kinds of cytokines and GFs participate this phase, such as transforming growth factor-bfamily (TGF-b, including TGF-b1, TGF-b2, and TGF-b3), interleukin (IL) Wound healing is an important but complicated process in human or animal, containing a multifaceted process governed by sequential yet overlapping phases, including hemostasis/ inflammation phase, proliferation phase, and remodeling phase.

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Many kinds of cytokines and GFs participate this phase, such as transforming growth factor-b family (TGF-b, including TGF-b1, TGF-b2, and TGF-b3), interleukin (IL) Wound healing is a complex and dynamic process Skin wounds are typically divided into acute and chronic wounds. Acute wounds are traumatic or surgical wounds that usually heal over time according to the normal wound-healing process (Raziyeva et al ;2021). Acute skin wounds vary from superficial scratches to deep wounds with variable amounts of tissue loss and damage to blood vessels, nerves, muscles or other tissues, or internal organs (Percival, 2002). Wounds are defined as chronic when they have failed to proceed through an orderly and timely process without establishing a sustained anatomic and functional result. They are not capable of closure or regaining the anatomy and function of healthy skin (Mustoe et al; 2002).

- Hibiscus rosa sinensis can be used as a diuretic.
- The fruits can be used in sprains, wounds etc [5,6].

Wound healing is a complex and dynamic process

The roots of Hibiscus rosa sinensis Linn can be used as a cough suppressant.

- Leaves and flowers can be used as a hair growth promoter and to prevent premature graying and to treat scalp
- Hibiscus rosa sinensis can be used as a diuretic.

- The fruits can be used in sprains, wounds etc [5,6].

The aim of the study was to develop and enhance hibiscus rosa sinensis extract nanoemulsion for wound healing in animal models.

2.Materials and Method

2.1.Materials

Hibiscus rosa sinensis, Liquid Paraffin, Abil EM 90 (Silicon co-polyol), Fragrance, Distilled water.

2.2.Plant materials

Fresh leaves of Hibiscus rosa sinensis were collected from different location of Dera Ismail Khan, Pakistan and were authenticated by Assistant professor Yasir Khan, Department of Botany, G.U.D.I.Khan. A voucher specimen (No.134582) has been deposited. The leaves were shade dried for 10 days and the dried leaves were then grinded in a grinder and stored in an air resistant container.

2.3.Extraction from Hibiscus rosa sinensis leaves

The leaves of Hibiscus rosa sinensis were properly cleaned in distilled water and left to dry in the shade for 10 days before being milled into a powder. Methanol and distilled water were used in an 80:33 (v/v) ratio for the extraction. The hydroalcoholic mixture was used to extract the powdered sample for one hour at 25 °C and 150 rpm in a 500 mL scale. The extracts were further dried in a CHILLER SLIM-CHIL-300 rotary evaporator set to low pressure and 35 °C after the mixture had been filtered using 125 mm Whatman filter paper. Ultimately, a light green residue was produced and kept in a cool, dark location in an airtight container (Ali et al; 2022 & Khoshrafta et al; 2019).

2.4.Preparation and optimization of Hibiscus rosa sinensis leaves extract based nanoemulsion

Using a magnetic stirrer, the components of the oil phase of abil EM-90 oil (1% *v/v*) and 1% *w/v* leaves extracts of Hibiscus rosa sinensis were set for spontaneous formation of small oil droplets that occur at the boundary between the aqueous and organic phases under certain system conditions. The mixture was first mixed for 15 min. When the extracts in the oil phase had completely dissolved, 1 combination of oil (Abil EM- 90 oil) and a mixture of surfactant (Liquid paraffin) was formed in different volume ratios to identify the stability differences. Low-energy approaches utilized the intrinsic properties of the emulsifier, oil, and water systems to form nanoemulsions (Khan et al; 2021). Thus, the extract in desired concentration was combined with the oil phase, and in other hand-distilled water, they were combined with a 2:1 ratio. With constant stirring, the aqueous phase was added drop by drop to the oil phase at 25 °C to produce the pre-emulsio. Then, high-shear homogenization (OV 625 Digital Disperser, Antylia Scientific, Monza, Italy) was used for 10 min at 10,000 rpm (Ullah et al; 2002).

2.5. Characterization of Hibiscus rosa sinensis leaves extract based nanoemulsion

Stability Study

The physical stability of the formulation under accelerated storage circumstances was ascertained by conducting a stability analysis

for the in situ formulation following the International Conference on Harmonization recommendations. The finished formulation was exposed to high temperatures and humidity levels. It was then stored for one to three months at various temperatures, including 4 °C ± 1 °C in the refrigerator, 22–25 °C ± 1 °C in the room, and 37 ± 2 °C in the incubator. Organoleptic alterations, physical stability, viscosity, and spreadability of the formulation were assessed at the end of the 30-, 60-, and 90-day periods (Sohail et al; 2018).

Physicochemical Properties and pH

Visual evaluations were conducted of the nanoemulsion's color, homogeneity, consistency, and phase separation (Le et al; 2023).Using a digital validated pH meter at room temperature, the pH of the prepared nanoemulsion was ascertained (Deore et al; 2019).

Particle Characterization

The produced nanoemulsion formulation's globule size was measured at 25 °C using Zetasizer ZTS1240 (Malvern Panalytical Ltd., Worcestershire, UK) (Deore et al; 2019).

Viscosity

A Brookfield rheometer was employed to assess the viscosity of the prepared nanoemulsion batches. Readings were taken at 100 rpm at 25 ± 2 °C (Jadhav et al; 2023).

FTIR Analysis

A Fourier transform infrared spectrophotometer (Nicolet 380 FT-IR Spectrometer, Madison, WI, USA) can be used for qualitative analysis and interaction investigations. This study aimed to evaluate the potential physiochemical interactions between the formulation ingredients used for preparing the nanoemulsion (Khan et al; 2022).

Spreadability of Nanoemulsion

The spreadability of the formulations was assessed using the Disc and Slip methodology (Latif et al; 2021) where two glass slides were used (7.5 cm long and 2.5 cm wide). The setup was composed of a wooden block with two glass slides (one slide is fixed, and the other one is movable) and a pulley fastened to a terminal. Subsequently, the formulation was mounted on the top fixed slide and pressed between the two plates to measure the spreadability of the mixture. To establish a homogenous layer of the formulation and release trapped air between the slides, the pulley's upper glass slide has a 100 g weight fastened to it. The duration required for the upper/top slide to move was then recorded. The spreadability of the nanoemulsion was calculated using the formula below:

$$S = M \times L/T \quad \text{(i)}$$

where spreadability is S, weight or mass placed on the movable slide is M, glass slide length is L, and time taken to cover distance is T.

Centrifugation

Formulations which were stable in the above test were centrifuged at 3600 rpm for 30min. Those formulations that did not show any phase separation were taken for freeze thaw stress test (Gurumukhi al; 2023).

Extract content

In this 2 ml of Nano emulsion was taken in 10 ml volumentric flask and the volume was

made up to 10 ml using methanol. 1ml of stock solution was diluted to 10 ml with phosphate buffer pH 6.0 phosphate buffer which was further diluted to give a final concentration of 10 µg/ml (10ppm) solution. Percent extract content was calculated spectrophotometrically at 234 nm (Gurumukhi et al; 2023).

Zeta Potential Determination

Zeta potential is a measurement of surface potential. The magnitude of zeta potential gives an indication of potential stability of an emulsion. Zeta potential is an important parameters in determining the stability of an emulsion and other colloidal dispersion, zeta potential larger than about 25mV is typically required to stabilize a colloidal system. Zeta potential is determined by a number of factors, such as the particle surface charge density, the concentration of counter ions in the solution, solvent polarity and temperature.Zeta potential can be determined using the Malvern Zeta sizer or the Nicomp particle sizer. Zeta potential is determined by electrophoretic light scattering(ELS).The smoluchowski equation can be used to compute the zeta potential from electrokinetic mobility µ (Gurumukhi et al; 2023).

µ=ζε/η.....equation. Where ε is the permittivity and η the viscosity of the liquid used

In Vitro Study

The release apparatus from Hanson Research was used for in vitro release. It had a working exterior area of 1.767 cm^2 and a receptor capacity of 7.0 mL. As the releasing membrane, a dialysis cellulose membrane was used. The membrane was allowed to equilibrate with the receptor medium as an equal volume of ethanol and distilled water (1:1 *v/v*) at pH 6.8 for one hour before 1 g of nanoemulsion was gently added to it (Kanekar, et al; 2024). A constant 37 ± 0.5 °C and 350 rpm were maintained for the releasing medium. At predefined intervals, after extracting the 2 mL sample, the same volume of media was added in its place (Ullah et al; 2023). At an effective wavelength of maximum absorbance of 234 nm, UV-visible spectrophotometry (UV-1900i, Shimadzu) was employed to estimate the extracted amount in every component (Scomoroscenco et al; 2021).

***In Vivo* Study**

Paravertebral incisions were made through the full thickness of the skin, 1 cm lateral to the midline of vertebral column after giving anaesthesia (Murthy et al; 2013). Rats were anaesthetized with ketamine and xylazine and an area of about ≈500 mm^2 was marked on the back of the rat by a standard ring. Changes in wound area were measured regularly and the rate of wound contraction calculated as given in the formula below.

%Wound contraction=1^{st} day reading-Last day reading / 1^{st} day reading X 100....(ii)

Statistical Analysis

All the results in tables and figures were used in triplicates and reported as averages ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied among all the measurements, and *p*-value ≤ 0.05 was considered statistically significant by using

SPSS 16.0 (IBM, USA) and Origin 2021 (Massachusetts, USA) software. Duncan and least significant difference (LSD) tests were calculated to measure the significance among the tested groups and properties.

3: Results and Discussion

3.1. Physicochemical evaluation of NE

The already prepared NE was kept at different temperatures (8°C, 25°C, and 40°C + 75 RH) for a duration of 28 days. Physical assessments of the formulation was carried out at predetermined intervals to check phase separation, consistency, liquefaction, odor, color change, and cracking. The freshly made formulations showed no phase separation after five minutes of centrifugation at 5000 and 10,000 rpm. When the pH of the early batch was measured, the findings showed a range of 5.5 to 6.5. According to study of Rehman et al. (2022), the pH of the formulation was within the range of human skin pH. After that, as Table 3.1 shows, a student t-test was used to look at the preparations' pH at various times, started on day 0 and checked every 7 days until day 28. There was no significant difference in the formulations' pH ($p > 0.05$). Topically applied formulations should have a pH of 5.5 to 6.5 to avoid skin irritation (Yeo et al., 2021). Over time, there may have been an unsure shift in pH due to the transport of water from one phase to another or the oil in the formulations producing acidic compounds (Burki et al., 2020). These pH fluctuations, though, fell within an acceptable range. Based on the previously listed factors, each preparation exhibited thermodynamic stability.

Table 3.1: ***pH of prepared NE at various conditions for 28 days.***

Time	**Temperature**		
	8 °C	**25 °C**	**40 °C**
	Formulation		
	NE	**NE**	**NE**
Day 0*	5.90	5.90	5.90
Day 7	5.85	5.85	5.70
Day 14	5.84	5.81	5.64
Day 21	5.80	5.77	5.61
Day 28	5.76	5.73	5.57

Day 0*: Indicates freshly prepared formulations.

3.2. Fourier transform infrared spectroscopic (FTIR) analysis

FTIR spectrum of hibiscus rosa sinensis has shown the characteristics peaks at 3390.61, 2922.22, 2854.00, 1640.22, 1460.69, 1376.85, 1259.69, and 1093.06 cm^{-1} shown in (Fig. 3.1) relate to the occurrence of strong N-O stretching, C=O stretching, C-H bending of alkane, and C-N stretching. The NE FTIR spectrum revealed that some peaks at 3353.71, 1614.59, 1398.63, 1336.35, 1240.20, 1058.97, 933.10, 898.56, 985.31 cm^{-1} ,included C=O stretching of carboxylic acid, C-H bending, and C=C bending. This can be observed in the

figure below. The FTIR analysis demonstrated that there is no interaction between the extract and the formulation's constituents in (Fig.3.2).

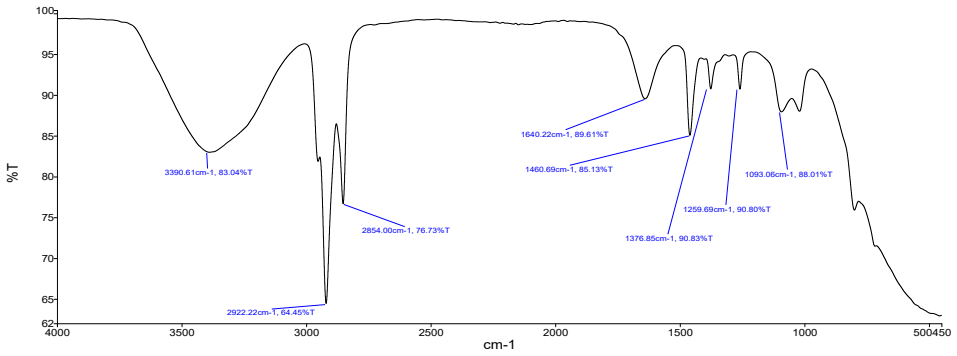


Fig. 3.1. FTIR spectrum of Hibiscus rosa sinensis extract

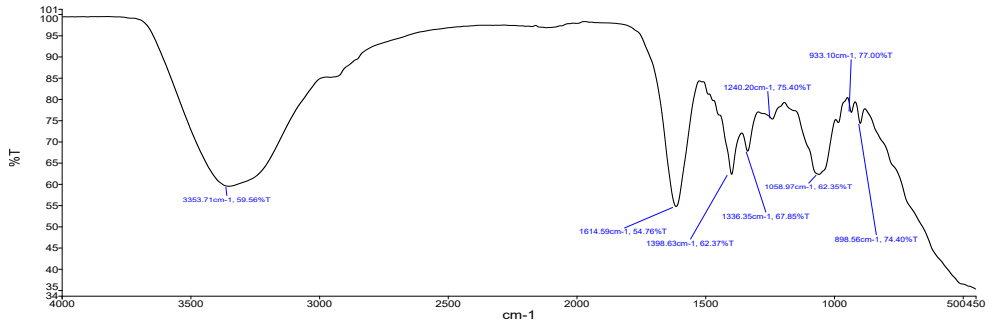


Fig. 3.2. FTIR spectrum of formulation

3.3. Viscosity of NE

Viscosities of hibiscus rosa sinensis loaded NE at various temperatures and intervals are displayed in Tables 3.2.

Table. 3.2: *iscosity of hibiscus rosa sinensis loaded NE at 8°C, 25°C and 40°C at various time intervals.*

Time	Viscosity (8 °C)		Viscosity (25 °C)		Viscosity (40 °C)	
	6 rpm	12 rp	6 rpm	12 rpm	6 rpm	12 rpm
Day 0	873	233	873	233	873	233
Day 07	870	221	870	219	850	216
Day 14	869	219	865	213	840	211
Day 21	866	216	860	208	835	206
Day 28	863	214	860	204	820	194

3.4. Zeta analysis

The formulation of topical preparation is significantly influenced by droplet size. In addition to size dispersion, the droplet size of the nanoemulsion affects hibiscus rosa sinensis extract release (Yeo et al., 2021; Nawaz et al., 2022). Table 3.4 shows the droplet size, zeta potential and poly dispersity index of formulations respectively.

Table. 3.4; *Droplet size, zeta potential and PDI of formulations.*

Formulations	Droplet size (nm)	Zeta Potential (mv)	PDI
Blank NE	53.71	−9.69	0.313
Extract loaded NE	66.83	−11.7	0.384

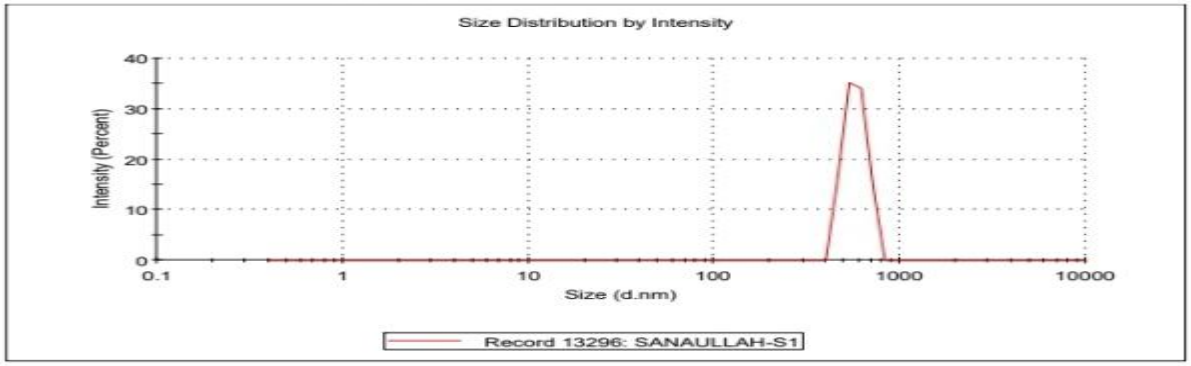


Fig. 3.3. Zeta analysis of formulation

3.5. Spreadability of NE

The ability of NE to disperse when used topically is referred to as their spreadability. The therapeutic efficacy of a topical preparation is determined by its spreadability (Ostertag & McClements, 2012). A minimum amount of shear is necessary for a preparation to have the best spreadability once it is taken out of the container (Akram et al., 2021). The spreadability coefficient of the topical preparation can be influenced by a number of variables, including high or low temperatures. It might be because the formulation becomes less spreadable at lower temperatures due to its increased viscosity. In a similar manner, Table 3.5 illustrates how increased temperature-induced decreases in viscosity enhanced spreadability. The relationship between viscosity and spreadability is inverse (Gharehbeglou et al., 2019).

Table. 3.5: *Average spreadibility values of formulations placed at 8°C, 25°C and 40°C.*

FORMULATIONS	SPREADABILITY (8 °C)	SPREADABILITY (25 °C)	SPREADABILITY (40°C)
BLANK NE	16	19	24
EXTRACT LOADED NE	15	18	21

3.6. Analysis of drug contents in the formulation

The percentage of extract in NE preparation indicates the manner in which extract is distributed throughout the preparation (Majid et al., 2021). The concentration of extract in NE was ±94.30%, which showed that the extract was distributed evenly throughout the

NE and the amount of extract was within the officially allowed range (i.e., ±10% allowed by the USP) of the Pharmacopeia (Hamid and Ali., 2020). These findings confirmed that every procedure followed was suitable for the multiple content assessments of the different preparation development.

3.7. *In-vitro* extract release

The amount of extract released from therapeutic dosage forms determines a drug's therapeutic properties. In the NE preparation, the percent extract release was determined to be 0 ±0%, 0.71 ±0.45%, 1.28 ±0.67%, 2.09 ±1.14%, 3.05 ±1.71%, 5.88 ±3.40%, 10.41 ±5.75%, 25.86 ±13.91%, 41.7±22.91% and 80.90±44.99% at the time intervals of 0min, 30mins, 1hr, 2hr, 4hr, 8hr, 12hr, 18hr, 20hr and 24h respectively.

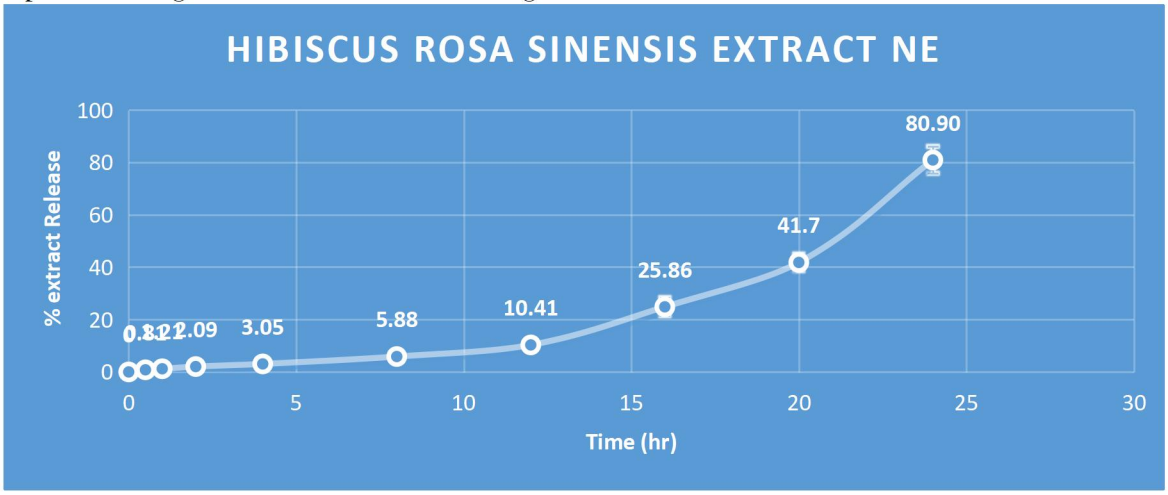
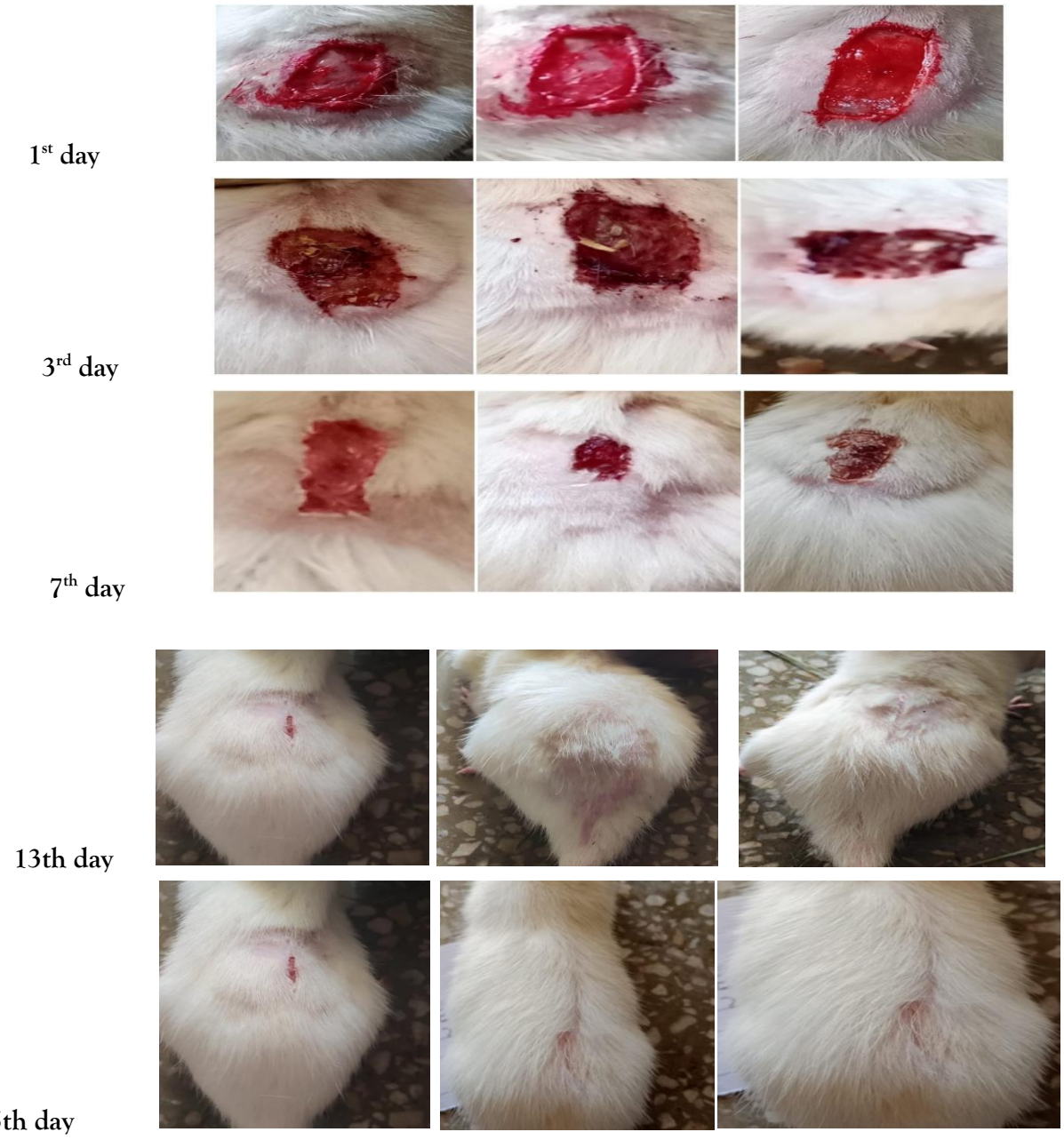


Fig.3.4. In-vitro release study of Hibiscus rosa sinensis extract NE

3.8. *In-vivo* wound healing activity

Topical drugs promote wound healing more than oral drugs because more of the drug is available at the site of injury (Mohamed et al., 2017). Hibiscus rosa sinensis effectiveness as a good release in topical drug delivery systems allows it to help speed up wound healing (Hajialyani et al., 2018). Figure shows the treatment progress of the groups. Day 15 wound contraction percentages for the standard and treated groups were 100% and 78%, respectively, for the positive control group. A one-way ANOVA was used to evaluate the data for each of the three groups, with a p-value of less than 0.05 indicating significance. The statistical analysis revealed that the wound-healing characteristics of the treated group were similar to those of Quench cream. It has been proposed that hibiscus rosa sinensis shows strong anti-bacterial activity against food-borne and human infections, which are significant contributors to the worsening of wounds after damage because of its good wound-healing activity (Sugumar et al., 2014). The results of our study showed that the treated group significantly recovered the wound and kept it stable in various storage conditions and temperatures.

Figure 3.5. In-vivo wound healing activity on Sprague Dawley rates



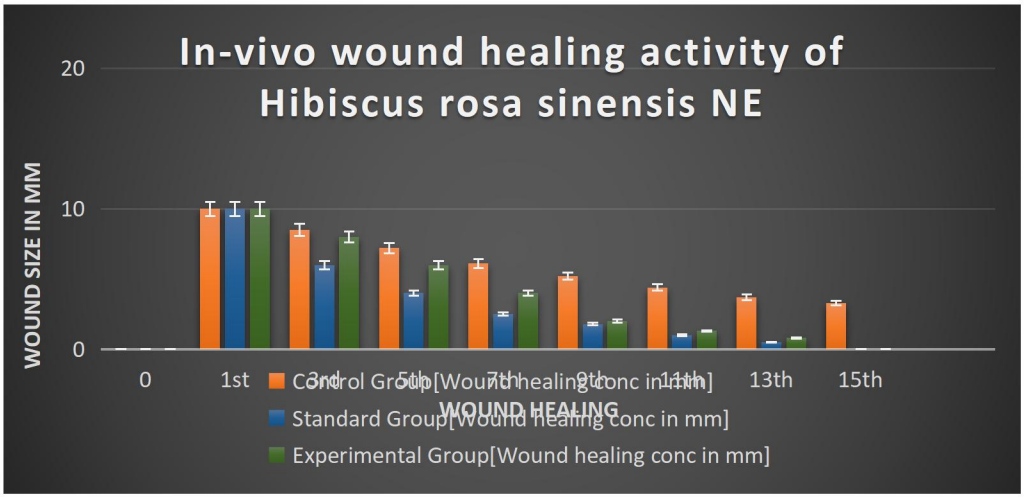


Fig.3.6. In-vivo wound healing activity of Hibiscus Rosa sinensis NE

4: Conclusion

The study concludes by developing and characterizing an hibiscus rosa sinensis extract loaded nanoemulsion for topical delivery, avoiding first pass effects, and preventing GIT irritation. Small droplet size, negative zeta potential, and PDI within the suggested range were all displayed by the enhanced formulation. The preparation was assessed utilizing a range of stability tests for 28 days. The results showed that the formulations were very homogeneous, thermodynamically stable, and free of phase separation. The pH was within a suitable range with only a small variation in temperature. Viscosity, spreadability, and drug content study results were all within an acceptable range. Animal studies have demonstrated the safety and efficacy of the topically applied hibiscus rosa sinensis extract loaded nanoemulsion formulation, which makes it the most effective alternative to oral drug delivery systems.

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