World Journal of Pharmaceutical and Life Sciences

Impact Factor: 8.21 www.wjpls.org Coden USA: WJPLA7

HERBAL LIPOSOMAL GEL OF *CENTELLA ASIATICA* EXTRACT FOR SCAR REDUCTION AND SKIN REGENERATION

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DOI: https://doi.org/10.5281/zenodo.20134041

How to cite this Article: Ratnapriya Singh[1]*, Mr. Mahadev Kanere[2], A. K. Singhai[3] (2026). Herbal Liposomal Gel Of Centella Asiatica Extract For Scar Reduction And Skin Regeneration. World Journal of Pharmaceutical and Life Sciences, 12(5), 335–343.

Article Received on 21/03/2026 **Article Revised on 11/04/2026** **Article Published on 01/05/2026**

ABSTRACT

Herbal liposomal gel formulations of *Centella asiatica* leaf extract were developed and evaluated for their potential in scar reduction and skin regeneration. The ethanolic extract of *C. asiatica* yielded a higher percentage (2.72%) of bioactive compounds compared to petroleum ether, and phytochemical analysis confirmed the presence of alkaloids, flavonoids, glycosides, phenolics, and triterpenoids, which are known to enhance wound healing and collagen synthesis. Liposomes were prepared and characterized, with formulation F3 exhibiting the smallest particle size (185.93 nm) and highest negative zeta potential (−31.8 mV), indicating optimal stability and skin penetration potential. The gel formulation displayed desirable physical properties, including smooth semi-solid consistency, off-white color, uniform homogeneity, pH 6.8, appropriate viscosity (4149 cps), and good spreadability (10.17 gm·cm/sec). Stability studies over 90 days showed minimal changes in pH and viscosity, demonstrating the robustness of the formulation. Overall, the *C. asiatica* liposomal gel represents a stable, effective, and biocompatible system with promising potential for topical application in scar management and skin regeneration.

KEYWORDS: *Centella asiatica*, liposomal gel, skin regeneration, phytochemicals, topical delivery, stability.

1. INTRODUCTION

Liposomes represent versatile Nano platforms for the improved delivery of pharmaceutical drugs and active compounds in a large variety of biomedical and nanomedicine applications **(Lombardo and Kiselev 2022).** They are characterized by easily controllable properties such as lipid composition, size, structure and morphology, surface charge, and the possibility of functionalizing their surfaces with polymers or ligands **(Díez-Pascual, 2022).** Particularly interesting is the ability of liposomal systems to encapsulate both hydrophilic and lipophilic active compounds as well as various biomolecules, including carbohydrates, proteins and peptides, DNA, or imaging compounds. Liposomes' structure is regulated by soft interactions and self-assembly phenomena that regulate their structural properties and stability within the environments of biological tissues **(Lombardo *et al.*, 2016).**

The inclusion of drugs within the vesicles' nanostructure favours the active compounds' solubilization in solution and protects against their chemical and biological degradation. Because of the unique characteristics of liposomes, including their colloidal stability, effective targeting, and site-specific delivery through a variety of administration routes, the use of liposome nanoformulation also results in a sensitive enhancement of their therapeutic performances. Of particular interest is the development of new liposome nano-platforms for biomedical and nanomedicine applications **(Karunaratne *et al.*, 2022).** The industrial applications of liposome Nano platforms include their use as drug delivery vehicles in nanomedicine, cancer, antimicrobial

therapy, as signal carriers in biomedical diagnostics and biochemistry, as adjuvants in vaccination, and as solubilizer and support matrices for various active compounds and macromolecules. Moreover, owing to their high biocompatibility and non-toxicity, liposomes are the most important category of clinically approved therapeutic drug nanocarriers for cancer treatment. Those systems play a crucial role also for the encapsulation of unstable bioactive substances (including antioxidants, antimicrobials, phytochemicals, and nutraceuticals) due to their strong enhancement of the colloidal stability **(Teixeira *et al.*, 2022).**

Centella asiatica forms mats of slender, creeping stolons that root at nodes. Its round to kidney-shaped leaves have scalloped edges and radiating veins, resembling small lily pads. Tiny pink to white flowers appear in clustered umbels near the soil surface, yielding minute reticulated fruits **(Sudhakaran, 2017).** The plant prefers semi-shade and waterlogged soils, making it common in rice paddies and along stream banks. It has been used to promote wound healing, enhance cognition, and reduce inflammation. Bioactive triterpenoids—especially asiaticoside and madecassoside—stimulate collagen synthesis and tissue repair, explaining its role in treating ulcers, burns, and varicose veins. In Ayurveda, it is classified as a *medhya rasayana*, a rejuvenating herb for the mind **(Bandopadhyay *et al.*, 2023).**

A herbal liposomal gel offers a synergistic approach by improving the delivery and efficacy of plant-derived bioactive compounds **(Kothapalli and Vasanthan, 2024).** The incorporation of *Centella asiatica* extract into a liposomal gel system can enhance its therapeutic performance by ensuring controlled release, targeted delivery, and prolonged retention at the site of application. This not only improves scar reduction but also supports skin regeneration and restoration of normal skin architecture **(Indriaty *et al.*, 2025).**

In this context, the present study focuses on the comparative phytochemical profiling and evaluation of antioxidant potential of Curcuma longa. The study involves extraction using different solvents, qualitative and quantitative phytochemical analysis, and assessment of antioxidant activity using established methods. The findings are expected to provide scientific validation for the traditional uses of the plant and support its potential application as a natural antioxidant source in pharmaceutical, nutraceutical, and functional food formulations.

2. MATERIALS AND METHIDS

2.1 Chemicals

Phosphatidylcholine, Cholesterol, Ethanol, Triethanolamine (TEA), Chloroform, Methanol, Asiaticoside / Madecassoside, Hydrochloric acid (HCl) and Sodium hydroxide (NaOH) were obtained from Merck, a reputable supplier of analytical reagents. HiMedia provided the Carbopol 934, Phosphate Buffer Saline (PBS) and Tween 80.

2.2 *Centella asiatica* plant Leaves Collection

Fresh, mature leaves of *Centella asiatica* were collected from healthy plants and washed with distilled water to remove impurities. The leaves were shade-dried at room temperature to preserve heat-sensitive phytochemicals, then finely powdered and stored in airtight containers under cool, dry conditions to maintain stability. Botanical authentication was carried out by a qualified taxonomist, ensuring the identity and quality of the plant material for further studies **(Ogunka-Nnoka *et al.*, 2020).**

2.3 Extraction Process and percentage yield determination

The leaves of *Centella asiatica* were shade-dried, powdered, and subjected to successive Soxhlet extraction. Petroleum ether was first used to remove non-polar constituents, followed by ethanol to extract polar phytochemicals such as flavonoids and phenolics. Each extraction was carried out for 6–8 hours until complete exhaustion. The extracts were then concentrated using a rotary evaporator at 40 °C, dried, weighed for percentage yield, and stored under suitable conditions for further analysis **(Sheneni *et al.*, 2018).**

2.4 Percentage Yield Determination

The dried extracts obtained from petroleum ether and ethanol were accurately weighed to determine the extraction efficiency. The percentage yield of each extract was calculated using the following formula.

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of plant material used (g)}} \times 100$$

The petroleum ether extract, representing non-polar constituents, and the ethanol extract, representing polar constituents, were separately assessed. The calculated yields provided an estimate of the amount of phytoconstituents successfully extracted from the *Centella asiatica* leaves and helped in selecting the appropriate extract for subsequent formulation and characterization studies. The extracts were also evaluated for basic organoleptic properties such as color, odor, and texture to ensure consistency and quality before further use **(Nofita *et al.*, 2022).**

2.5 Preformulation studies

2.5.1 The Organoleptic Studies

The organoleptic characteristics of the *Centella asiatica* leaf extract were assessed using standard visual and sensory evaluation techniques. Parameters including color, odor, texture, and general appearance were carefully observed **(Kumalasari *et al.*, 2024).**

2.5.2 Solubility study

The qualitative solubility of *Centella asiatica* leaf extract was evaluated in accordance with the methods described in the Indian Pharmacopoeia. A measured amount of the

extract was placed in separate 10 mL test tubes and tested for solubility in different solvents, including methanol, DMSO, distilled water, chloroform, and acetone (1 mL each). The ability of the extract to dissolve in each solvent was observed and recorded to provide insight into its solubility profile, which could guide further formulation development **(Jäger *et al.*, 2007).**

2.6 Quantitative Estimation of Phytoconstituents

2.6.1 Total Phenolic Content (TPC) Estimation

The total phenolic content of *Centella asiatica* leaf extract was determined using the Folin–Ciocalteu colorimetric method. A series of standard solutions of gallic acid were prepared to generate a calibration curve. The extract was mixed with Folin–Ciocalteu reagent and sodium carbonate solution, then incubated at room temperature for a specified period. The absorbance of the resulting blue-colored solution was measured using a UV-Visible spectrophotometer at 760 nm. The phenolic content in the extract was expressed as milligrams of gallic acid equivalent per gram of dried extract (mg GAE/g), calculated from the standard calibration curve **(Sánchez-Rangel *et al.*, 2013).**

2.6.2 Total Flavonoid Content (TFC) Estimation

The total flavonoid content was estimated using the aluminum chloride colorimetric method. Standard solutions of rutin were prepared to construct a calibration curve. The extract was mixed with a specific volume of 2% aluminum chloride solution and incubated for a fixed time at room temperature. The formation of yellow complex was measured spectrophotometrically at 510 nm. The flavonoid content of the extract was expressed as milligrams of rutin equivalent per gram of dried extract (mg RE/g), calculated from the standard curve **(Shraim *et al.*, 2021).**

2.7 Preparation of Liposomes formulation

Several parameters influence the final properties of liposomes. Major variables in the liposome properties include cholesterol and lecithin amounts and vortex time. Five different formulations with low and high values of cholesterol (50 and 250 mg), phospholipid (100 to 500 mg), and vortex time (15 minutes) were used to prepare liposomal formulations. Liposomes were prepared by thin film method. Briefly, different concentrations of soya lecithin and cholesterol (Table 1) were dissolved in the chloroform-methanol (1:1) and 500 mg extract was added to the solution, then the mixture was evaporated in a rotary evaporator when the thin film was formed in the round-bottoms flask, it was hydrated with phosphate buffer (pH 7.4). The suspension was agitated by vortex for 30 minutes and then sonicated for one hour **(Moghimipour *et al.*, 2015).**

Table 1: Composition of Liposome Formulations (F1–F5)

Ingredients	F1	F2	F3	F4	F5
***Centella asiatica* Extract (mg)**	500	500	500	500	500
Phospholipid (mg)	100	200	300	400	500
Cholesterol (mg)	50	100	150	200	250
Phosphate buffer pH 7.4 (mL)	10	10	10	10	10
Chloroform (mL)	5.0	5.0	5.0	5.0	5.0
Methanol (mL)	5.0	5.0	5.0	5.0	5.0
Methyl paraben (%)	0.02	0.02	0.02	0.02	0.02
Propyl paraben (%)	0.01	0.01	0.01	0.01	0.01

2.8 Characterization of liposomes formulation

2.8.1 Physical properties

Visual inspection was used to assess the prepared liposome formulations' physical characteristics. Color, homogeneity, clarity, and the existence of any visible aggregates or sedimentation were among the parameters that were closely monitored **(Maritim *et al.*, 2021).**

2.8.2 Size Distribution or Particle size

The particle size of the prepared liposome formulations was measured using a Malvern Zetasizer through Dynamic Light Scattering **(Elorza *et al.*, 1993).**

2.8.3 Zeta potential

The surface charge of the prepared liposome formulations was determined using a Malvern Zetasizer **(Soema *et al.*, 2015).**

2.8.4 Scanning Electron Microscopic (SEM)

The surface morphology of the optimized liposomal formulation was analyzed using Scanning Electron Microscopy (SEM). A small amount of the sample was placed on a metal stub, air-dried, and coated with a thin layer of gold or palladium to enhance conductivity. The sample was then examined under high vacuum, where an electron beam generated high-resolution images of the liposomes. SEM analysis provided detailed information on vesicle shape, size, surface characteristics, and any aggregation, aiding in the characterization of the formulation **(Bibi *et al.*, 2011).**

2.8.5 Formulation of liposomal gel

Carbopol-934 was first dispersed in 50 mL of warm water and allowed to hydrate for 2 hours. The dispersion was then stirred uniformly using a magnetic stirrer at 600 rpm. In a separate container, carboxymethyl cellulose and methyl paraben were dissolved in another 50 mL of warm water with continuous stirring to form

homogeneous solution. Both dispersions were then combined while stirring continuously. Triethanolamine was added dropwise to adjust the pH and achieve the desired gel consistency. The prepared liposomes were incorporated into this mixture, followed by the propylene glycol as a permeation enhancer. The final blend was stirred thoroughly until a smooth, lump-free gel was obtained **(Wasankar *et al.*, 2012).**

Table 2: Composition of liposomal gel formulation.

Name of Ingredient	Gel Formulation
Carbopol 934	1.0 gm
Carboxymethyl cellulose	1.0 gm
Propylene glycol	0.5 ml
Methyl paraben	0.2 ml
Liposome	10 ml
Triethanolamine	q.s
Water	50 ml

2.9 Characterization of extract loaded liposomal gel formulation

2.9.1 Physical appearance

The physical appearance of the *Centella asiatica* extract-loaded liposomal gel was assessed visually. Parameters such as color, homogeneity, consistency, and presence of any lumps or phase separation were carefully observed (**Singh *et al.*, 2014).**

2.9.2 Measurement of pH

The pH of the *Centella asiatica* extract-loaded liposomal gel was determined using a calibrated digital pH meter **(Buck *et al.*, 2002).**

2.9.3 Determination of Viscosity

The viscosity of *Centella asiatica* extract-loaded liposomal gel was measured using a Brookfield viscometer at room temperature **(Wasankar *et al.*, 2012).**

2.9.4 Spreadability of extract loaded liposomal gel

A topical gel that is placed or rubbed over the skin's surface should have high spreading coefficient. About 1 g of formulation was put on a glass slide to evaluate this. The gel was sandwiched between the two glass slides and distributed at a predetermined distance by placing a 50 mg bulk on top of another glass slide that was the same length as the first one. It was noted how long it took the gel to move a specific distance from its starting point.

The spread ability was determined by following formula.
$S = M \times L/T$

Where, S-Spread ability, g.cm/s M-Weight put on the glass slide's upper L-length T-Time for spreading gel in sec **(Ramesh and Kumar, 2017).**

2.10 Stability study of liposomal gel formulation

The stability of the *Centella asiatica* extract-loaded liposomal gel formulation was evaluated over a period of 90 days. The formulation was filled into suitable, airtight containers and stored under different conditions, including room temperature (25 ± 2 °C), refrigerated conditions (4 ± 2 °C), and accelerated conditions (40 ± 2 °C with 75% RH). Stability evaluations were carried out at predetermined time intervals of 0, 30, 45, 60, and 90 days. At each interval, the formulation was examined for changes in pH and spreadability. The observations recorded at each time point were compared with the initial values (day 0) to identify any significant variations. This stability study was conducted to confirm that the liposomal gel maintained its physical integrity, consistency, and suitability for topical application throughout the storage period **(Dragicevic-Curic *et al.*, 2010).**

3. RESULTS AND DISCUSSION

3.1 Plant Collection

Table 3: Plant collection.

Plant name	Plant part used	Weight
Centella asiatica	Leaves	400 gm

3.2 Percentage Yield

The percentage yield of the extract was calculated to determine the efficiency of the extraction process. It was obtained by comparing the weight of the dried extract with the initial weight of the plant material used. The percentage yield provides an indication of how effectively the bioactive compounds were extracted and helps in evaluating the suitability of the extraction method employed.

Table 4: Percentage Yield of crude leaves extracts of *Centella asiatica* extract.

Plant name	Solvent	Color of extract	Theoretical weight	Yield(gm)	% yield
Centella asiatica	Pet ether	Greenish brown	400	3.85	0.96 %
	Ethanol	Yellowish brown	385	10.51	2.72%

3.3 Organoleptic properties

Table 5: The Organoleptic Studies of *Centella asiatica Leaves* extract.

Parameters	*Centella asiatica*
Colour	Yellowish brown
Odour	Characteristic
Appearance	Semi-solid / viscous

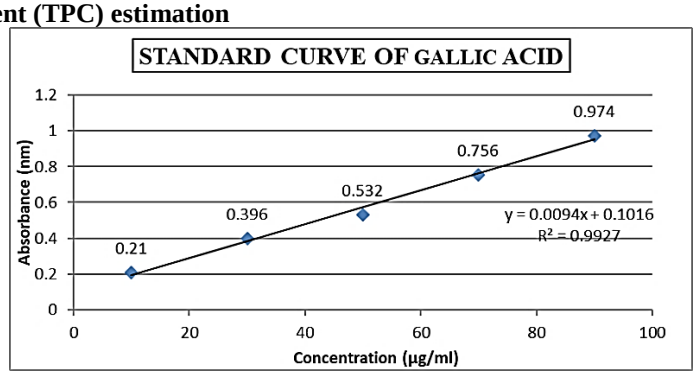
3.4 Preliminary Phytochemical study

Table 6: Phytochemical testing of *Centella asiatica* leaves extract.

Experiment	Presence or absence of phytochemical test	
	Pet. Ether extract	Ethanolic extract
Alkaloids		
Dragendroff's test	Present (+)	Present (+)
Mayer's reagent test	Present (+)	Present (+)
Wagner's reagent test	Present (+)	Present (+)
Hager's reagent test	Present (+)	Present (+)
Glycoside		
Borntrager test	Absent (-)	Present (+)
Killer-Killiani test	Absent (-)	Present (+)
Carbohydrates		
Molish's test	Absent (-)	Present (+)
Fehling's test	Absent (-)	Present (+)
Benedict's test	Absent (-)	Present (+)
Barfoed's test	Absent (-)	Present (+)
Iodine Test	Absent (-)	Present (+)
Flavonoids		
Shinoda Test	Present (+)	Present (+)
Tannin and Phenolic Compounds		
Ferric Chloride test	Present	Present (+)
Lead Acetate Test	Present (+)	Present (+)
Gelatin Test	Present (+)	Present (+)
Saponin		
Foam test	Present (+)	Present (+)
Froth Test	Present (+)	Present (+)
Test for Triterpenoids and Steroids		
Salkowski's test	Present (+)	Absent (-)
Libbermann-Burchard's test	Present (+)	Absent (-)

3.5 Quantitative Estimation of Phytoconstituents

3.5.1 Total Phenolic content (TPC) estimation

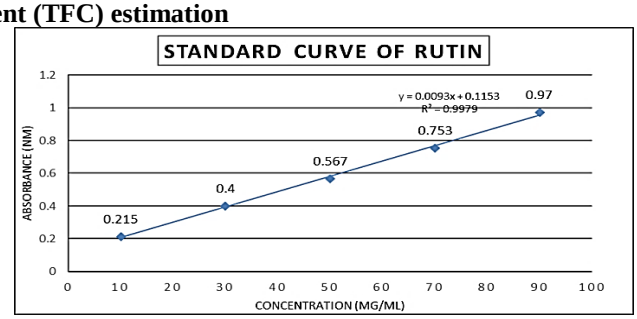


Graph 1: Represent standard curve of Gallic acid.

Table 7: Total Phenolic Content in *Centella asiatica* extract.

Absorbance	TPC in mg/gm equivalent of Gallic Acid
0.205	12.66 mg/gm
0.215	
0.226	

3.5.2 Total Flavonoids content (TFC) estimation



Graph 2: Represent standard curve of Rutin.

3.5.2.1 Total Flavonoid Content

Table 8: Total Flavonoid Content in *Centella asiatica* extract.

Absorbance	TFC in mg/gm equivalent of Rutin
0.210	11.74 mg/gm
0.221	
0.234	

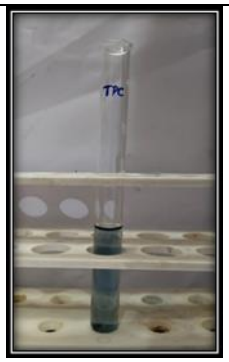
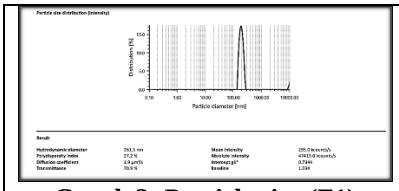


Figure 1: Total Phenolic Content.

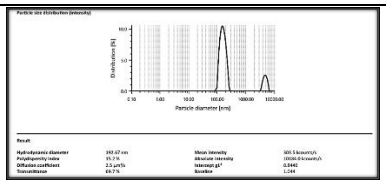
Figure 2: Total Flavonoid Content.

3.6 Evaluation parameter of liposomes formulation

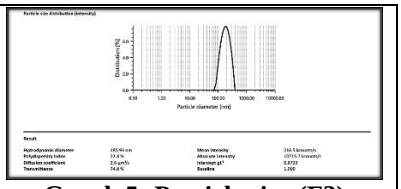
3.6.1 Particle size



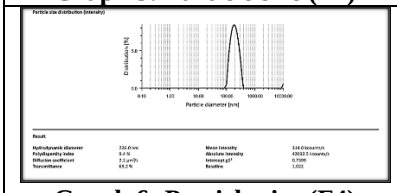
Graph 3: Particle size (F1)



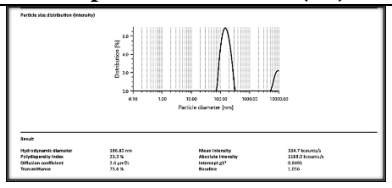
Graph 4: Particle size (F2)



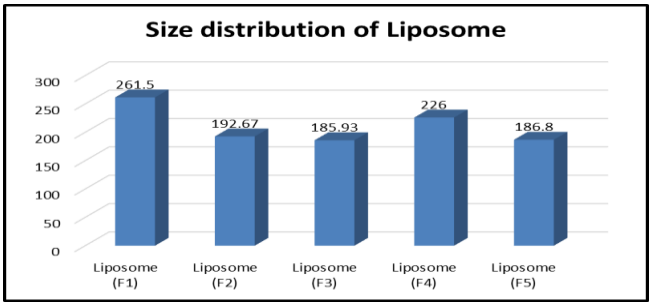
Graph 5: Particle size (F3)



Graph 6: Particle size (F4)

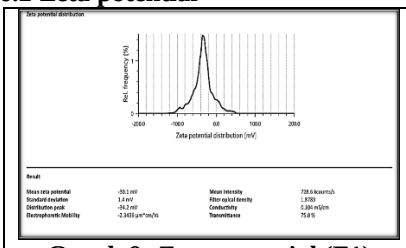


Graph 7: Particle size (F5)

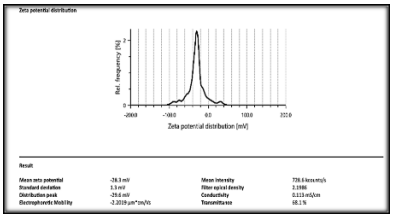


Graph 8: Graphical representation of Size distribution of Liposome.

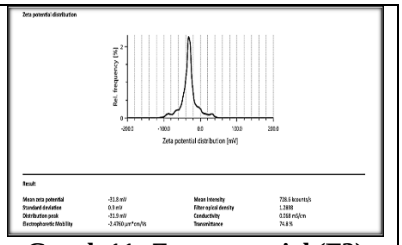
3.6.2 Zeta potential



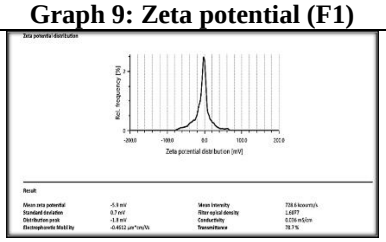
Graph 9: Zeta potential (F1)



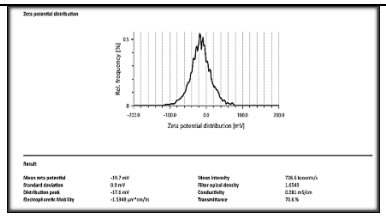
Graph 10: Zeta potential (F2)



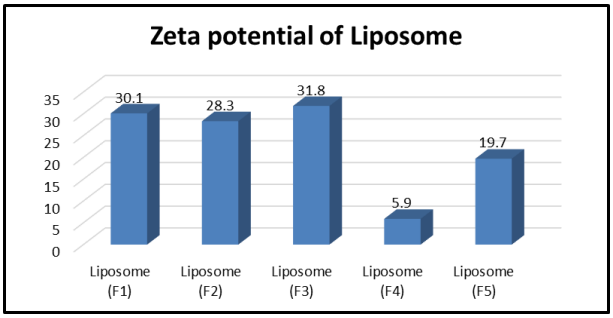
Graph 11: Zeta potential (F3)



Graph 12: Zeta potential (F4)



Graph 13: Zeta potential (F5)



Graph 14: Graphical representation of Zeta potential of Liposome.

3.6.3 Scanning Electron Microscopic (SEM)

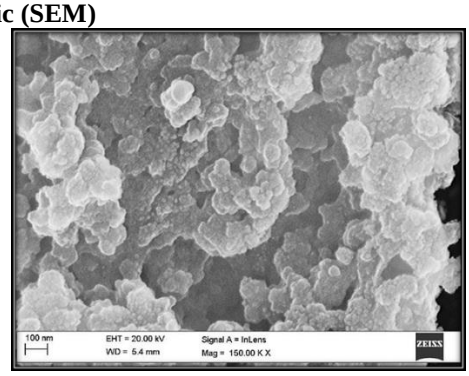


Figure 27: SEM (F3).

3.7 Evaluation parameter of Liposomal gel formulation

3.7.1 Physical properties

Table 9: Physical properties of Liposomal Gel.

Parameters	Results
Physical appearance	Smooth, semi-solid gel
Colour	Off-white to pale yellow
Homogeneity	Uniform, no visible lumps or phase separation

3.7.2 Measurement of pH, Viscosity and Spreadability

Table 10: pH, Viscosity and Spreadability of Liposomal Gel.

Formulation	pH	Viscosity (cps)	Spreadability (gm.cm/sec)
Liposome (F3)	6.8	4149±0.26	10.17

3.8 Stability study

Table 11: Stability Study of liposome gel formulation.

Time (Days)	25 °C ±2 °C & 60 ± 5% RH		40 °C ±2 °C & 70 ± 5% RH	
	pH	Viscosity (cps)	pH	Viscosity (cps)
0	6.8	4149	6.8	4149
30	6.8	4135	6.7	4100
45	6.8	4130	6.6	4065
60	6.7	4125	6.5	4030
90	6.7	4120	6.4	4000

DISCUSSION

The study demonstrated that the leaf extract of Centella asiatica possesses promising physicochemical and biological characteristics suitable for topical formulation. Ethanol proved to be a more efficient solvent than petroleum ether, yielding a higher amount of extract rich in polar phytoconstituents such as phenolics and flavonoids. The extract showed acceptable organoleptic properties, good solubility in multiple solvents, and moderate levels of total phenolic and flavonoid content, supporting its antioxidant potential. The optimized liposomal formulation (F3) exhibited desirable properties, including small particle size (<200 nm), high zeta potential indicating stability, and suitable physical characteristics such as appropriate pH, viscosity, and spreadability. The liposomal gel was homogeneous, non-irritating, and stable under both standard and accelerated conditions with only minor variations over time. Overall, these findings confirm that the developed liposomal gel of *Centella asiatica* is stable, effective, and suitable for topical application, with potential benefits in skin regeneration and scar management.

4. CONCLUSION

The study successfully formulated a stable herbal liposomal gel containing *Centella asiatica* extract with promising characteristics for scar reduction and skin regeneration. The extraction and phytochemical evaluation confirmed the presence of therapeutically relevant compounds, and the optimized liposomal formulation (F3) demonstrated superior size and stability attributes essential for effective topical delivery. The gel's physicochemical parameters including appropriate pH, consistent viscosity, good spreadability, and stability over time — indicate a formulation well suited for skin application without irritation while ensuring retention of functional activity Coupled with the well-documented biological actions of *Centella asiatica* such as stimulation of fibroblast activity, collagen synthesis, anti-inflammatory effects, and enhanced healing dynamics, this formulation holds promise for improving scar appearance and supporting regenerative processes in skin. Therefore, the developed herbal liposomal gel represents a scientifically substantiated topical system with potential efficacy for clinical or cosmeceutical use in scar management and dermal repair.

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