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Research Article

Formulation and Evaluation of Liposome Loaded Anti-Aging Cream

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ABSTRACT

Aim: The current study's objective was to formulate and evaluate a liposome loaded anti-aging cream in order to improve the absorption and to show long lasting effect. Tinospora cordifolia and Centella asiatica was incorporated into the liposome which shows antioxidant and anti-inflammatory activity. Liposome delivery system was preferred due to its greater solubility, permeability, and bioavailability. It carries drug which shows increased drug penetration. Materials and methods: Liposome were prepared using ethanol injection method. Soya lecithin act as main phospholipid and stabilize the formulation. Cholesterol provides stability as well as it controls permeability which prevents leak out of encapsulated drug. Liposome was incorporated into cream base. Evaluation was carried out for both liposomes include zeta potential and FESEM & cream include physical appearance, homogeneity, pH, spreadability, viscosity, and washability. Results And Discussion: FTIR studies shows that the extracts and excipients were compatible with each other. Zeta potential indicates that the liposome has minimal aggregation with consistent size distribution and FESEM confirms the successful formation of liposome (particle size 141.6 nm and 176.2 nm). The cream shows white and homogenous with a soft and smooth texture. It was easily washable and spreadable. Antioxidant studies confirmed that the cream showed strong antioxidant activity. Conclusion: Liposome loaded anti-aging cream provides an effective and biocompatible platform for controlled and targeted dermal delivery of plant-derived compounds, improving therapeutic performance and cosmetic benefits.

INTRODUCTION

Creams are the formulation that are generally employed on the surface of skin typically appearing as viscous emulsions or semisolid blend of oil in water or water in oil.[1] Herbal cream has been employed for centuries for natural remedies for various skin disease and cosmetic purposes. Plant based ingredients are utilized for formulation of herbal cream to protect, maintain and to restore the properties of skin.[2] Creams are infused with

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herbal extracts that provide antioxidant properties and act as a natural defense, these ingredients neutralize free radicals, soothe inflammation and enhance the skin's natural healing mechanism.[3]

Antioxidants are the molecules which are capable of neutralizing the free radicals by giving or receiving electrons to eliminate unpaired electrons. Natural compounds like phenolics (phenols and polyphenols), flavonoids, carotenoids, steroids, and thiol compound show antioxidant property.[4]

Antioxidants show protection to biological system by two mechanisms:

1. They react directly with free radicals and neutralizing it by donating the electrons thereby it reduces the oxidative reaction.
2. They remove the reactive oxygen and nitrogen species before these produce damage to the biological system.[5]

Aging of the skin is marked by a rise in wrinkles, age spots, dryness, thinning, and a loss of elasticity. The process of skin aging is influenced not only by internal factors but also by external environmental elements like UV radiation, air pollution, smoking, and poor nutrition, all of which can speed up skin aging and increase the number of senescent cells present in the skin.[6]

Intrinsic aging of skin refers to the deleterious mechanisms that occur over time in the three layers of tissue. Many theories attempt to explain the aging and one of those theories states that aging process is a consequence of multiple damages that accumulate at the intra and extracellular levels, including damage to the genome or epigenome, telomere shortening, loss of proteostasis, and even mitochondrial dysfunction.[7]

Physiological aging occurs as the body grows older, the extracellular matrix of the skin gradually deteriorated. This natural breakdown weakens the skin's structural support, resulting in thinner and more delicate skin leads to visible appearance of wrinkles.[8]

Liposomes are small, globular vesicles comprises of one or more concentric lipid bilayers enclosing an internal aqueous compartment. These membranes are made from natural or synthetic lipids, and due to its unique bilayer structure, liposomes are widely used for entrapping hydrophilic and lipophilic substances.[9] The name "Liposome" was taken from the Greek words "Lipo" that denotes as "fat" whereas "soma" indicated body, meaning lipid-based body or structure.[10] These liposomes were initially developed in year 1961 by Alec D. Bangham.[11]

This study explores the formulation of liposome loaded anti-aging cream utilizing *Tinospora cordifolia* and *Centella asiatica*, which are known for its antioxidant, anti-inflammatory and immunomodulatory properties. When compared to conventional formulation, topical liposomal formulations can be less harmful, more effective and show long lasting effects.

MATERIALS AND METHODS

2.1 DRUGS AND CHEMICALS

Active ingredients

- *Tinospora cordifolia*
- *Centella asiatica*

Other excipients

- Cholesterol
- Soya Lecithin
- Phosphate buffer
- Borax

- Liquid paraffin
- Bees wax
- Methyl paraben
- Propyl paraben
- Rose oil
- Distilled water

2.2. Selection of plant

Tinospora cordifolia (family *Menispermacea*) and *Centella asiatica* (family *Umbellifereae*) were gathered from the neighborhoods of Bengaluru, Karnataka.

The fresh leaves of *Tinospora cordifolia* and *Centella asiatica* were collected. They were identified and authenticated by taxonomist at Central Ayurveda Research Institute, Bengaluru, Karnataka.

2.3. Extraction of *Tinospora cordifolia*

The leaves of *Tinospora cordifolia* were cleaned and dried into small pieces and pulverized in blender. The powder was stored in a container at room temperature. 100 g of powder was extracted using the repeated maceration method with 50% methanol (v/v) in macerator for three days. The filtrate was then filtered and evaporated using rotary vacuum evaporator. Further the extract was collected, weighed and refrigerated until further processing.[12]

Fig 1: Extract of *Tinospora cordifolia*

2.4. Extraction of *Centella asiatica*

The leaves were cleaned, shade dried and pulverized in blender. The powder was stored in an air tight container at room temperature. 100 g of powder was extracted using maceration method with 50% methanol (v/v) in macerator for three consecutive days. The filtrate was then filtered and evaporated using rotary vacuum evaporator. Further the extract was collected, weighed and refrigerated until further processing.[13]

Fig 2: Extract of *Centella asiatica*

2.5. FTIR studies

FTIR studies were conducted to determine the drug's compatibility with the excipients.

2.6. Formulation of liposome

- Liposome was formulated by using ethanol injection method.
- In this method appropriate quantity of extracts (*Tinospora cordifolia* & *Centella asiatica*), phospholipids, and cholesterol were dissolved in ethanol which acts as the organic phase and phosphate buffer of pH 6.8 will be aqueous phase.
- Using a syringe, the organic phase was introduced into the aqueous phase dropwise while the aqueous phase was kept on magnetic

stirrer and maintained the temperature of 55ºC and 600 rpm.

- As soon as the ethanolic solution came into contact with the aqueous phase, spontaneous liposome formation occurred.
- After sometimes, ethanol is evaporated and liposomal suspension was formed.
- Further this suspension is dried and incorporated into cream.[14]

Fig 3: Liposome preparation

2.7. Formulation of liposome loaded anti-aging cream

- The water in oil type of cream was prepared using 2 phases.
- All the active pharmaceutical ingredients and excipients are measured accurately.
- The oil phase was prepared by melting bees wax along with liquid paraffin by maintaining the temperature to 70ºC.
- Similarly, aqueous phase was prepared by heating borax, methyl paraben, and propyl paraben with sufficient amount of distilled water by maintaining the temperature to 70ºC.
- When the two phases are attained 70ºC temperature, aqueous phase is generally introduced into the oil phase with continuous stirring until the cream consistency is attained.
- Once the temperature gets reduced the prepared liposome suspension was incorporated to the cream base by slow stirring.
- Rose oil was added to the formulation as a perfume and cream is stirred well.[15]

Fig 4: Formulation of cream

Table 1: Composition of cream (10 g)

INGREDIENTS	F1	F2	F3	F4
LIPOSOME	0.5 g	1.0 g	1.25 g	1.5 g
BORAX	0.2 g	0.2 g	0.2 g	0.2 g
BEES WAX	3.0 g	3.0 g	3.0 g	3.0 g
METHYL PARABEN	0.05 g	0.05 g	0.05 g	0.05 g
PROPYL PARABEN	0.02 g	0.02 g	0.02 g	0.02 g
LIQUID PARAFFIN	2.8 g	2.8 g	2.8 g	2.8 g
ROSE OIL	3 drops	3 drops	3 drops	3 drops
DISTILLED WATER	q.s	q.s	q.s	q.s

2.8. Evaluation of the formulation

Evaluation parameters of Liposome

2.8.1. Zeta potential determination for particle size

Zeta potential is a measure of the magnitude of the electrostatic charge repulsion or attraction between particles, and is one of the fundamental parameters known to affect stability in terms of dispersion, aggregation or flocculation, and can be applied to improve the stability of formulations of dispersed systems.

The sample was analyzed by Zetasizer Nano ZS (Make: microtrac, Model: Nanotrac-USA). The stock solution of nanomaterials is prepared at a concentration of 10 mg/ml in double distilled water was ultrasonicated using ultrasonic bath for 5 min, followed by transferring the same into the liquid cell and measured for the particle size and zeta potential. Further the stability of nanomaterials was evaluated by using zeta potential analysis.

2.8.2. Field emission scanning electron microscopy (FESEM)

A drop of liposome (liquid form) was spread on conducting carbon tape which was already pasted on aluminum stub (base) of FESEM (TESCAN, Model: Mira3) chamber. The stub was kept in oven at 100ºC for 10 hours to ensure total moisture removal. The image was captured using electron detector which detects the secondary electron emitted from sample when high energy electron beam with high voltage (kV) strikes on sample surface.

Evaluation parameters of Cream

2.8.3. Physical appearance

The physical appearance of the cream can be observed by its color, roughness, and was graded.

2.8.4. Homogeneity

The formulation was tested for the homogeneity by visual appearance and by touch.

2.8.5. Washability

Washability test was carried out by applying small amount of cream on slide then washing with tap water.

2.8.6. pH of the cream

The pH meter was calibrated using standard buffer solution. About 0.5 g of cream was weighed and dissolved in 50 ml of distilled water and its pH was measured using digital pH meter.

2.8.7. Spreadability

The spreadability of cream formulation was determined by using parallel plate method. An adequate amount of sample is taken between two glass slides and a weight of 10 g is applied on the slides for 5 minutes.

Spreadability can be expressed as,

$$S = \frac{m \times l}{t}$$

where,

- S = Spreadability.
- m = weight applied to upper slide.
- l = length moved on the glass slide.
- t = time taken.

2.8.8. Viscosity

The viscosity of all the semisolid preparation was measured by using Brookfield viscometer. Approximately around 10 g of sample was taken in a sample holder. The temperature was maintained at around 25 ± 0.5ºC. Further a constant rpm of 20 was maintained for viscosity measurement. The spindle was fixed to the viscometer head and allowed to rotate as specified at 20 rpm. Over a period of 60 sec till a constant reading was displayed on the viscometer screen. The readings were tabulated and reported.

2.8.9. Antioxidant activity (DPPH Assay)

The DPPH scavenging assay is a simple chemical experiment for the primary evaluation of any compound for its simplicity and low cost for free radical scavenging activity.

The cream's free radical scavenging ability was tested using DPPH solution at 517 nm.

DPPH [1,1-diphenyl-2-picryl hydrazyl] is a stable free radical with purple color. Antioxidants reduce DPPH to 1,1-diphenyl-2-picryl hydrazine, colorless compound which is measured at an absorbance of 517 nm.

Preparation of 0.1 mM DPPH Solution:

Accurately weigh 3.6 mg of DPPH and transfer in to a 1000 ml of volumetric flask, add methanol to dissolve DPPH completely and make up to the mark with methanol.

Sample and control solution preparation:

Prepare different concentration of ascorbic acid and cream at varying concentration in methanol (1, 2, 4, 8, 16, 32, 64 µg/ml).

For the control, replace the test sample with 2 ml of methanol.

Procedure:

Up to 3 ml of ethanolic DPPH solution was added with the 0.5 ml sample solution. At 517 nm DPPH and sample shows its absorbency was taken by UV spectrophotometer after 30 minutes and then comparison was made between the absorbance of a sample and the absorbance of ascorbic acid (standard).

Then, the percentage inhibition was calculated by the following equation:

$$Scavenging\ activity\ (\%) = \frac{(A\ Control - A\ Sample)}{A\ Control} \times 100$$

where,

- A Control = Absorbance of DPPH solution without sample
- A Sample = Absorbance of DPPH solution with the test sample

IC50 is the concentration of the sample required to inhibit 50% of DPPH radicals. Plot % scavenging vs. concentration and calculate the IC50 using regression analysis.

RESULTS AND DISCUSSION

3.1. FTIR studies

FTIR studies of herbal extracts (*Tinospora cordifolia* and *Centella asiatica*) and excipients was performed as shown in figure since, all the functional groups were intact. It was found that the drug and excipients were compatible with each other.

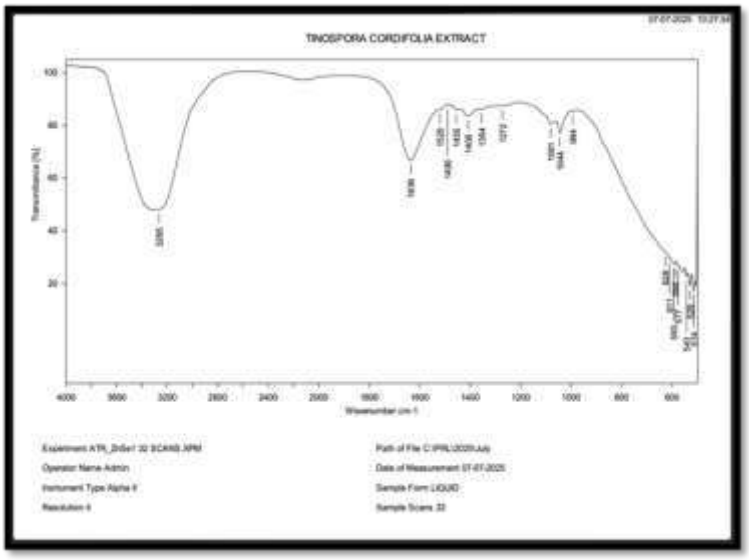

Fig 5: FTIR report for *Tinospora cordifolia*

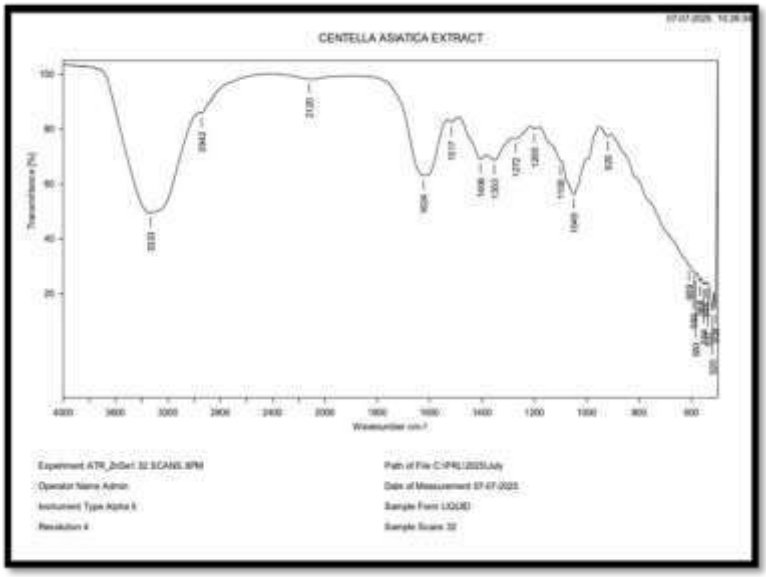

Fig 6: FTIR report for *Centella asiatica*

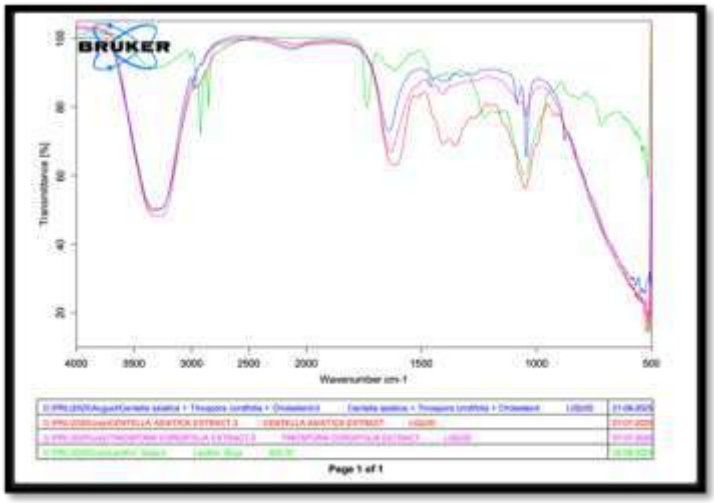

Fig 7: FTIR report for comparison

3.2. Zeta potential determination for particle size

The particle size distribution graph indicates a narrow and consistent distribution within the nanometer range. The peak occurs near 100 nm, suggesting most particles are around this size. The sharp peak and steep cumulative curve imply uniform particle size and minimal aggregation. Such a distribution is ideal for applications requiring controlled nanoscale properties, such as cosmetics, coatings, or pharmaceutical formulations.

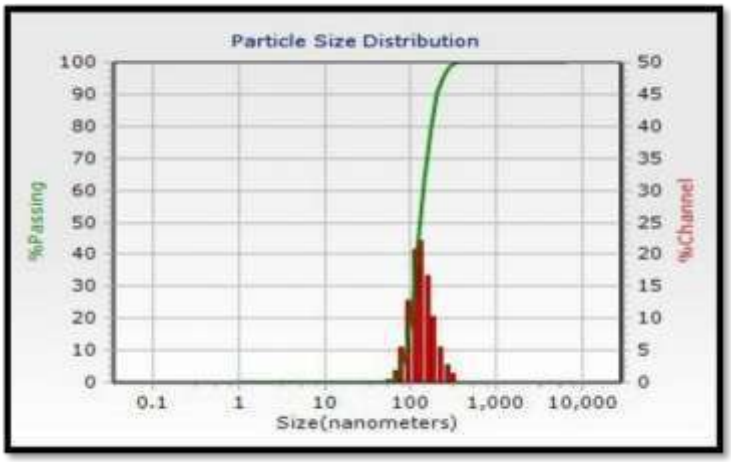

Fig 8: Particle size distribution

Interpretation:

- Dominant particle size range: ~ 80-120 nm
- Mean particle size: ≈ 100 nm
- Distribution type: Narrow, unimodal
- Dispersion quality: Good (minimal clumping observed).

Conclusion: The sample demonstrates a well-dispersed nanoparticle system with consistent size distribution suitable for industrial or research applications requiring nanoscale uniformity.

3.3. Field emission scanning electron microscopy (FESEM)

The particle size of formulated liposome was found to be 141.6 nm and 176.2 nm.

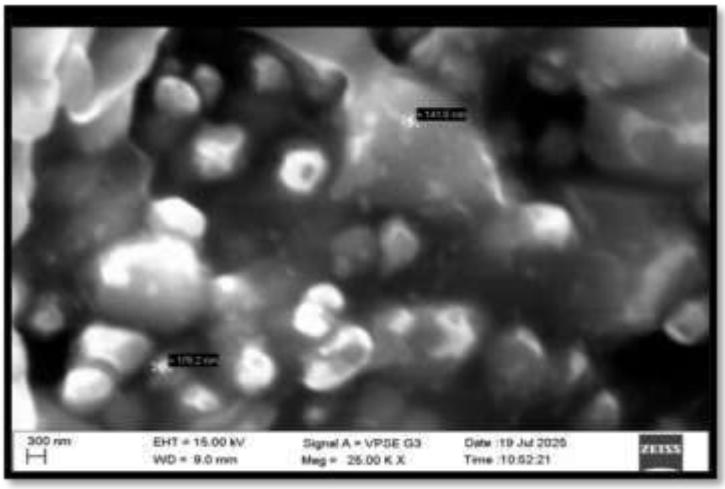

Fig 9: FESEM results conforming presence of liposomes at 300nm

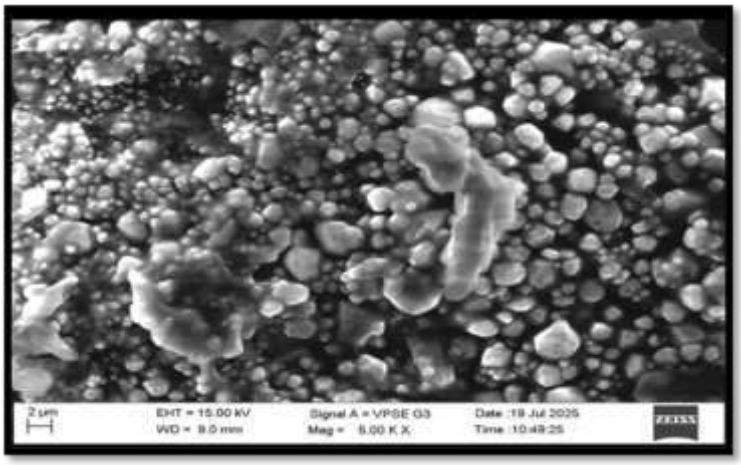

Fig 10: FESEM result conforming presence of liposomes at 2µm

The FESEM images confirms the successful formation of liposome. The observed spherical morphology and particle sizes (ranging from 300 nm to 2 µm) indicate efficient encapsulation and distribution of liposomes within the cream. This confirms the stability and integrity of liposomal vesicles in the prepared formulation, suitable for enhanced drug delivery and skin penetration.

3.4. Physical appearance

The prepared cream was white and homogenous with a smooth and soft texture. The cream was easily spreadable.

Table 2: Organoleptic parameters

Formulation (F)	Appearance	Colour	Odour	Texture
F1	Clear	White	Pleasant	Soft
F2	Clear	White	Pleasant	Soft
F3	Clear	White	Pleasant	Soft and smooth
F4	Clear	Buff colour	Pleasant	Soft

Fig 11: Physical appearance of cream

3.5. Homogeneity

All formulations show uniform distribution of liposome in cream. This was confirmed by visual appearance and by touch. The appearance and touch of the cream was good.

3.6. Washability

All formulation shows easy washable when the cream on slide was washed with tap water.

Table 3: Washability parameter

Formulation (F)	Washability
F1	Washable
F2	Washable
F3	Washable
F4	Washable

3.7. pH of the cream

pH of cream was determined using digital pH meter and found to be in the range from 6.1 to 6.4, indicating that the cream is non-irritating and suitable for topical application.

Table 4: pH of cream

Formulation (F)	pH of the cream
F1	6.42
F2	6.20
F3	6.13
F4	6.46

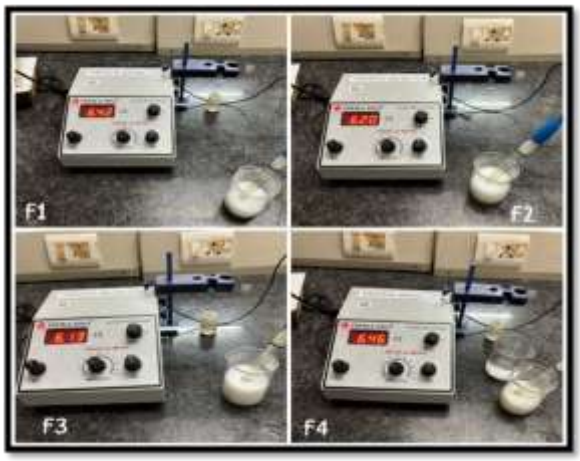

Fig 12: pH of cream formulation

3.8. Spreadability

The spreadability value for the formulation was found to be in the range 55-70 g.cm/5 min.

Table 5: Spreadability of cream

Formulation (F)	Spreadability (g.cm/5min)			Average Spreadability
	Trial 1	Trial 2	Trial 3	
F1	60	62	60	60.67
F2	68	72	66	68.67
F3	70	68	72	70.00
F4	56	52	58	55.33

Fig 13: Spreadability of cream formulation

3.9. Viscosity

Viscosity of cream was measured using Brookfield Ametek carrying spindle no. as T/F. The viscosity of cream can generally fall between approximately 1500 cP to 120,000 cP.

Table 6: Viscosity of cream

Trial No	Name of the sample	Speed (RPM)	Temperature (°C)	Viscosity (cP)
Trial 1	Cream	20	24.7	79,200
Trial 2			25.2	80,100

3.10. Antioxidant activity

The antioxidant activity of liposome loaded antiaging cream was assessed using DPPH radical scavenging activity and by taking ascorbic acid as standard.

Formulation shows maximum percentage inhibition of about 93.8% (640 µg/ml)

The results were compared with a standard antioxidant (Ascorbic acid) to determine the effectiveness of the formulation in neutralizing free radicals.

IC_{50} was calculated by plotting graph between concentration and %RSA.

Table 7: Calculation of % RSA and IC 50 from DPPH Assay of Ascorbic acid

Calculation of % RSA and IC 50 from DPPH Assay				
Conc (µg/ml)	**Control**	**Sample**	**% RSA**	**IC 50 (µg/ml)**
0	0.650	0.650	0	**16.7**
1	0.650	0.540	16.9	
2	0.650	0.430	33.8	
4	0.650	0.300	53.8	
8	0.650	0.180	72.3	
16	0.650	0.090	86.2	
32	0.650	0.040	93.8	
64	0.650	0.010	98.5	

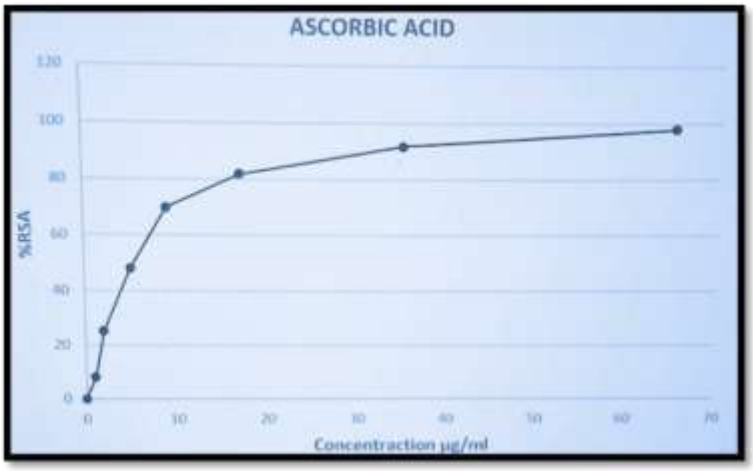

Fig 14: Interpretation of results of ascorbic acid

Table 8: Calculation of % RSA and IC 50 from DPPH Assay of Cream

Calculation of % RSA and IC 50 from DPPH Assay				
Conc (µg/ml)	**Control**	**Sample**	**% RSA**	**IC 50 (µg/ml)**
0	0.650	0.650	0	**127.08**
10	0.650	0.600	7.7	
20	0.650	0.540	16.9	
40	0.650	0.470	27.7	
80	0.650	0.325	50.0	
160	0.650	0.180	72.3	
320	0.650	0.090	86.2	
640	0.650	0.040	93.8	

Fig 15: Interpretation of results of cream

CONCLUSION

The formulation and evaluation of liposome loaded anti-aging cream using *Centella asiatica* and *Tinospora cordifolia* was successfully formulated and evaluated. The cream was formulated as water-in-oil (W/O) emulsion, ensuring ease of application and better skin compatibility. Physicochemical evaluations confirmed its white and homogeneous with a soft and smooth texture. The cream was easily

spreadable, easily washable, and appropriate pH (6.13).

The antioxidant study revealed that the cream exhibited significant free radical scavenging activity (93.8% inhibition), indicating strong anti-aging potential. The presence of bioactive compounds from *Centella asiatica* and *Tinospora cordifolia* contributed to the cream's ability to protect against oxidative stress, a key factor in skin aging.

The present study highlights the potential of herbal ingredients in skincare, promoting both cosmetic needs and skin health. The successful formulation of the liposome loaded anti-aging advances the integration of traditional herbal knowledge with modern cosmetic science, encouraging further exploration of natural resources. The results encourage more research into herbal cosmetics and the use of other natural resources to improve skincare. The creation of this liposome cream is a step forward in combining traditional herbal knowledge with modern cosmetic science.

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