WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 9, 1571-1590. Research Article ISSN 2277–7105

FORMULATION AND DEVELOPMENT OF HERBAL ETHOSOMAL GEL FOR ANTI-AGING PROPERTY USING ROSEMARY (SALVIA ROSMARINUS) AND KIWI (ACTINIDIA DELICIOSA) EXTRACT

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Article Received on
21 March 2025,

Revised on 11 April 2025,
Accepted on 01 May 2025

DOI: 10.20959/wjpr20259-36244

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ABSTRACT

This study focuses on the development, characterization, and optimization of an ethosomal formulation designed for anti-aging applications. The formulation consists of Rosemary and kiwi extracts, soy lecithin, and propylene glycol, incorporated into ethosomes and further integrated into Carbopol 934K gel for enhanced topical delivery. Five ethosomal formulations were prepared with varying ingredient concentrations to identify the optimal formulation. The evaluation involved determining entrapment efficiency, particle size, zeta potential, and polydispersity index (PDI). Transmission electron microscopy (TEM) and atomic force microscopy (AFM) were used to analyze surface morphology, while viscosity, pH, spreadability, and extrudability were assessed to confirm the gel's suitability for topical application. Stability studies examined ethosomal formulations under different conditions over a two-month period. Among the formulations, EF1 emerged as the most optimized, showing an entrapment efficiency of 89.48 ± 0.43%, PDI of 0.14 ± 0.05, zeta potential of 47.7 ± 0.5 mV, and vesicle size of 139.7 ± 1.55 nm. The ethosomal suspension and lyophilized ethosomal suspension demonstrated the best stability at 4°C/60 ± 5% RH. This study concludes that the ethosomal formulation of Rosemary and kiwi extracts provides a synergistic anti-aging effect. The ethosomal system enhances penetration, prolongs drug retention, and ensures targeted and sustained release. With optimized ingredients and processing conditions, the study demonstrates that ethosomal gel formulations are a promising strategy for advanced anti-aging topical treatments.

KEYWORDS: *Ethosomes, Rosemary extract kiwi extract, Anti-Aging, Dermal, Transdermal*

delivery.

INTRODUCTION

Topical drug delivery is one of the more advanced methods for delivering drugs. It is advantageous because it delivers the medication via the skin at a controlled and predefined rate by passive diffusion, which maximizes systemic impact. Drug distribution is regulated using transdermal patches with hydrophilic and lipophilic polymers. For lipid-based systems, ethosomes are essentially new, creative, and intrusive carrier vesicles that were initially described by Touitou et al. (2000). Their sophisticated and improved drug delivery mechanism is intended for them because of their pliable, soft, and flexible nature. Essentially, these are phospholipid vesicles, and their applications are in transdermal and cutaneous drug delivery systems. They primarily contain ethanol, water, and several concentric layers of flexible phospholipids; in the case of liposomes, however, cholesterol is included in place of ethanol. This bilayer vesicular system (aqueous and lipid) exhibits attraction for both lipophilic and hydrophobic drugs, hence augmenting their bioavailability. The high percentage of ethanol in it, compared to liposomes, enhances medication administration topically and helps to weaken the lipid bilayer. Consequently, in contrast to liposomal drug delivery systems, it directs penetration into deep layers of the skin by increasing permeation through the stratum corneum barrier. This is not simply made possible by the way ethanol acts on the stratum corneum; vesicle permeability and deformability also play a part. Furthermore, its combination with skin lipids enhances skin penetration, leading to a steady-state transdermal flow of about 1 mg/cm²/h. As of yet, the process by which drugs enter the skin through ethosomes remains unclear. However, a high ethanol content, phospholipid- and skin-lipid-containing vesicles, and deep penetration of medication into the skin layers through the ethosome transport system are associated with this phenomenon. Ethanol and lipid molecules both improve fluidity in the polar head region, which increases membrane permeability. Owing to their unique properties, ethosomes are regarded as superior carriers for transdermal medication delivery systems and are acknowledged as an improved liposome. Previous research has shown that ethosomes improve the transdermal delivery of various active compounds in both in-vitro and in-vivo studies.[1]

Kiwi extract, known for its rich antioxidant content, and rosemary extract, recognized for its anti-aging properties, were used in this study to develop a nanosized ethosomal gel for enhanced skin penetration. Since ethosomal gels are highly effective with minimal adverse

effects, they increase the permeability of active compounds via the skin due to enhanced transdermal flux, thereby improving bioavailability. Using ethanolic rosemary extract, an ethosomal system was developed to deliver kiwi extract as the active anti-aging agent. The drug-loaded ethosomes were then incorporated into a Carbopol gel, ensuring sustained transdermal delivery. The formulation underwent multiple in-vitro characterization tests, including assessments of particle size, zeta potential, PDI, entrapment efficiency, TEM, AFM, pH, viscosity, Spreadability, and extrudability. These evaluations confirmed the efficacy and suitability of the formulation for anti-aging treatment, demonstrating its potential as an advanced topical delivery system.[2][3]

MATERIALS AND METHOD

Materials

Table 01: List of materials.

Sr. No.	Ingredients	Company Name
1.	Kiwi fruit	Sabji Mandi, Bhujpura, Aligarh.
2.	Rosemary	Green India Nursery, Aligarh.
3.	Soya lecithin	R.K. Medical Agency, Aligarh.
4.	Propylene glycol	R.K. Medical Agency, Aligarh.
5.	Carbopol 934K	R.K. Medical Agency, Aligarh.
6.	Tri-ethanol amine	R.K. Medical Agency, Aligarh.
7.	Glycerine	R.K. Medical Agency, Aligarh.
8.	Ethanol	R.K. Medical Agency, Aligarh.
9.	Xanthan gum	R.K. Medical Agency, Aligarh.

Preparation of kiwi extract

To extract kiwi juice and active compounds, start by washing, peeling, and cutting the fruits into small pieces before crushing them into a puree. Transfer the puree into a clean glass jar and add ethanol in a 1:1 or 2:1 ratio to help preserve the bioactive compounds. Ensure the kiwi is fully submerged, seal the jar tightly, and store it in a cool, dark place for 1-2 weeks, shaking it daily to enhance extraction. After the steeping period, strain the kiwi-ethanol mixture into a clean container, pressing the pulp to extract as much liquid as possible. Transfer the strained extract into a dark glass bottle to protect it from light. Properly stored, the ethanolic kiwi extract remains stable for several months to a year.[4][5]

Preparation of rosemarry extract

To extract rosemary oil, start by thoroughly washing and drying fresh rosemary or drying it directly. Chop the rosemary into small pieces and place them in a clean glass jar, ensuring it is completely submerged in ethanol. Seal the jar and store it in a cool, dark place for 2 to 6 weeks,

shaking it daily to enhance oil extraction. After the steeping period, strain the mixture through cheesecloth or a fine strainer into a clean bowl. Transfer the strained ethanol mixture into a shallow dish and leave it in a well-ventilated area at room temperature to allow ethanol to evaporate. This process may take several days to a week, depending on temperature and airflow. Ensure the dish is in a warm place away from open flames. Once the ethanol has fully evaporated, collect the concentrated rosemary oil and store it in a small, dark glass bottle. When stored properly, the oil remains usable for 6 months to a year.[6][7]

Formulation of kiwi Extract and Rosemary extract loaded ethosomes

Kiwi extract and rosemary extract loaded ethosomes were prepared using Touitou's method, with ingredients weighed in different concentrations. Kiwi extract, Rosemary ethanolic extract, phospholipids, and other components were dissolved in ethanol and mixed vigorously in a covered vessel at room temperature. The resulting mixture was then heated to 30°C using a water bath, while water was separately heated to the same temperature and gradually added to the mixture. This solution was continuously stirred at 700 rpm in a sealed vessel using a magnetic stirrer. To enhance ethosome stability and uniformity, the formulation was stored at 4°C and underwent three cycles of sonication using a probe sonicator, each lasting 5 minutes, with a 5-minute interval between cycles.[8][9]

Table 02: Composition of Kiwi and Rosemary extract loaded ethosomes formulation (EF) with soya-lecithin.[10]

Composition	EF1	EF2	EF3	EF4	EF5
Kiwi Extract (ml)	3	3	3	3	3
Rosemary Extract (ml)	3	3	3	3	3
Soya-Lecithin (gm)	1.5	2	1.5	2	1.5
Propylene glycol (gm)	3	2	3	3	2
Ethanol (ml)	4.5	6	4	5	4
Glycerine (ml)	2	-	3	-	-
Xanthan Gum (gm)	-	-	-	0.3	-
Water	q.s.	q.s.	q.s.	q.s.	q.s.

Kiwi Extract and Rosemary Extract loaded ethosomes incorporated into gel

Carbopol 934K (0.75% w/v) was dissolved in distilled water and stirred with a magnetic stirrer for one hour to ensure proper dispersion. As the polymer swelled, 20 mL of Kiwi and Rosemary extract-loaded ethosomal suspension was added, and the mixture was continuously stirred at 30°C and 700 rpm until a uniform ethosomal gel was formed. To adjust the pH to the desired range, triethanolamine was added. A total of six ethosomal formulations were

prepared and evaluated based on various parameters.[11][12][13][14]

Characterization of Kiwi and Rosemary extract loaded ethosomes

Determination of size of the Particle, polydispersity index (PDI) and zeta potential

Determining key parameters such as particle size, polydispersity index (PDI), and zeta potential is crucial for evaluating ethosomal formulations. These measurements were conducted using the Malvern Zeta Sizer Nano ZS, which operates on the principle of dynamic light scattering (DLS). To begin the analysis, 1 mL of the ethosomal mixture was diluted with High- Performance Liquid Chromatography (HPLC) water. The measurements were taken at a 90° scattering angle, with a medium viscosity of 0.8862 cP and a refractive index of 1.36, ensuring accurate characterization of the formulation's stability and dispersity.[15]

Structural study of Kiwi and Rosemary extract loaded ethosomes by Transmission electron microscopy (TEM)

The morphology of the formulation was analyzed using Transmission Electron Microscopy (TEM) to assess its internal structure, shape, and crystallization. For the experiment, 20 mL of the formulation was diluted with deionized water and stained with 2% w/v phosphotungstic acid for 30 seconds. The prepared specimens were then placed on a copper-laminated grid for drying, with two grids prepared for each sample to ensure accuracy and reproducibility.[16]

Structural study of Kiwi and Rosemary extract loaded ethosomes by Atomic force microscopy (AFM)

The study utilized Atomic Force Microscopy (AFM) to analyze the ethosomal suspension. A 1 mL sample was placed on a mica sheet and incubated for 5 minutes. Any unattached ethosomes were then removed by washing the sample with deionized (DI) water. After air drying at room temperature, further scanning was conducted using the Advanced Integrated Scanning Tool for Nanotechnology (AIST-NT), Model: Smart SPM 1000. Each AFM image was displayed separately, showing the cantilever’s phase, amplitude, and height signals in the trace direction, providing detailed structural and morphological insights.[17]

Determination and calculation of Drug Entrapment efficiency, *EE (%)*

Entrapment efficiency (EE) is used to determine the percentage of the drug encapsulated within a colloidal particle system. To measure EE, a small amount of the ethosomal formulation is placed in an Eppendorf tube and centrifuged at 14,000 rpm for 15 minutes. The

supernatant is then carefully collected using ultracentrifugation with a TLA-45 rotor, allowing for the precise quantification of the unencapsulated drug.[18]

For detecting the drug's concentration at 299nm, a UV/Visible spectrophotometer is used. The formula is used to determine the total amount of drug trapped in the system pf colloidal particles.

$$\mathbf{EE(\%) = \frac{(quantity\ of\ incorporated\ drug - quantity\ of\ free\ drug\ present\ in\ the\ superanatant)}{quantity\ of\ incorporated\ drug} \times 100}$$

Determination of pH, viscosity, Spreadability and Extrudability of Gel

- The pH of the prepared ethosomal gel was measured using a digital pH meter, with the anode and cathode of the glass electrode immersed in the formulation. The readings were then recorded and analysed.
- For viscosity measurement, a Brookfield viscometer was used. The instrument was immersed in a beaker containing the ethosomal gel, and viscosity was measured at different speeds while maintaining a constant room temperature throughout the process.
- After precisely weighing an ethosomal gel, it was sandwiched between two 8cm long slides of glass. The time needed to pull the top slide and extend the gel to lower slide was calculated with various weights applied to pulley. The reading was taken three times and lastly measurements for Spreadability was calculated from the following formula-

S = M* L/T

Where

S = denote the ethosomal gel spredability

M = denote weight applied to the top slide (g)

L = indicate the distance the slide has moved (cm)

T = indicate the time taken by the top slide to move downwards (sec)

- In this study, 20g of Kiwi and Rosemary extract-loaded ethosomal gel was packed into a flexible tube. Pressure was applied while a clamp was attached to prevent backflow. The amount of gel released was measured and recorded until the pressure stabilized, ensuring consistency in the formulation's extrudability.[19]

Determination of stability studies

The stability of an ethosomal formulation depends on the lipid layer's ability to retain and release drugs over time. To evaluate this, stability testing was conducted for two months at different temperatures. Both lyophilized ethosomes and ethosomal suspensions were stored separately and analyzed after 7, 15, 30, 60, and 90 days. The findings emphasize the significance of drug retention capacity in ethosomes, which plays a crucial role in their long-term stability and effectiveness.[20][21]

***In vitro* study for skin permeability**

The in vitro dermal absorption method involves applying a test formulation, which may be radiolabelled, to a skin sample acting as a barrier between the donor and receptor compartments of a diffusion cell. There are two types of diffusion cells: static and flow-through. Static diffusion cells collect and replace perfusate at set intervals, while flow-through cells continuously circulate it using a pump. Static cells can be oriented horizontally or vertically— horizontal cells are commonly used for general skin absorption studies, while vertical (side-by- side) cells are preferred for advanced drug delivery techniques like sonophoresis, iontophoresis, and electroporation.

This technique requires submerging both surfaces of the skin sample, which may lead to excessive hydration and potential skin damage. To ensure accurate results, diffusion cells must be made from inert, non-adsorbing materials, with receptor chamber capacities ranging from 0.5 to 10 mL and an exposed membrane surface area of 0.2 to 2 cm^2. A minimum of six skin samples should be used for testing.

In the Franz diffusion cell setup, the prepared skin sample was heated to room temperature, and the receptor compartment was filled with 10 mL of pH 7.4 phosphate buffer. A semi-permeable membrane filter was placed at the bottom of the donor section, ensuring proper separation. The system was maintained at 37 ± 1°C using a magnetic stirrer set at 200 rpm for uniform mixing.

To begin the experiment, 1 g of Kiwi and Rosemary extract-loaded ethosomal gel was accurately weighed and placed in the donor compartment on the membrane filter. At specific time intervals (1, 2, 3, 4... up to 24 hours), 1 mL aliquots were collected from the receptor chamber and replaced with fresh media to maintain sink conditions. The collected samples were then filtered through a 0.45 µm membrane and analyzed using a UV/visible spectrophotometer at 299 nm against an appropriate blank.

This procedure was repeated for all formulations, and a graph was plotted to show the percentage cumulative drug release over time. Each ethosomal formulation was tested three times, and the results were reported as mean ± SD to ensure reliability.[22][23]

RESULTS

Characterization of Kiwi and Rosemary extract Loaded Ethosomes

Determination of size of the Particle, polydispersity index (PDI) and zeta potential

The particle size was determined using dynamic light scattering (DLS), and the results for both particle size and PDI are shown in Table No. 03. The ethosomal formulation has a range of particle sizes from 139.7 ± 10.55 to 231.8 ± 12.43 nm, while the PDI ranges from 0.114 ± 0.05 to 0.348 ± 0.08. In comparison to the other preparations, formulation EF1 has the highest entrapment effectiveness, with an optimum particle size and PDI of 139.7 ± 10.55 nm and 0.114 ± 0.05, respectively.

The greatest values for particle size and PDI, on the other hand, are 231.8 ± 12.43 nm and 0.348 ± 0.08, respectively, in formulation EF5. Vesicle size plays a significant role in the delivery of topical medications. The vesicle becomes smaller the more successfully it transports the material into the skin's underlying layers. The particle's sizes were arranged as follows: EF1< EF3< EF2< EF4< EF5.

In contrast, the sequence of PDI was EF1< EF3< EF4< EF2< EF5. Zeta potential is considered while evaluating the stability of the ethosomal formulation.

The zeta potential values varied from -17.5 ± 0.8 to 47.7 ± 0.5 mV, as shown in Table No. 03. The zeta potential value of EF1 is the highest at 47.7 ± 0.5 mV, whereas that of EF4 is the lowest at roughly -31.1 ± 0.5 mV. According to Verma et al. (2003), zeta potential can be found in the following order: EF4< EF5< EF3< EF2< EF1 (du Plessis et al., 1994).

Table 03: Characterization of different ethosomal formulation.

Characterization	EF1	EF2	EF3	EF4	EF5
Particle size	139.7 ±10.55	160.6 ±11.20	158.1 ±19.40	187.3 ±08.80	231.8 ±12.43
PDI	0.114 ±0.05	0.259 ±0.05	0.194 ±0.02	0.209 ±0.02	0.348 ±0.08
Zeta potential	47.7 ± 0.5	-17.5 ± 0.8	-23.9 ± 0.6	-31.1 ± 0.5	-25.5 ± 0.3

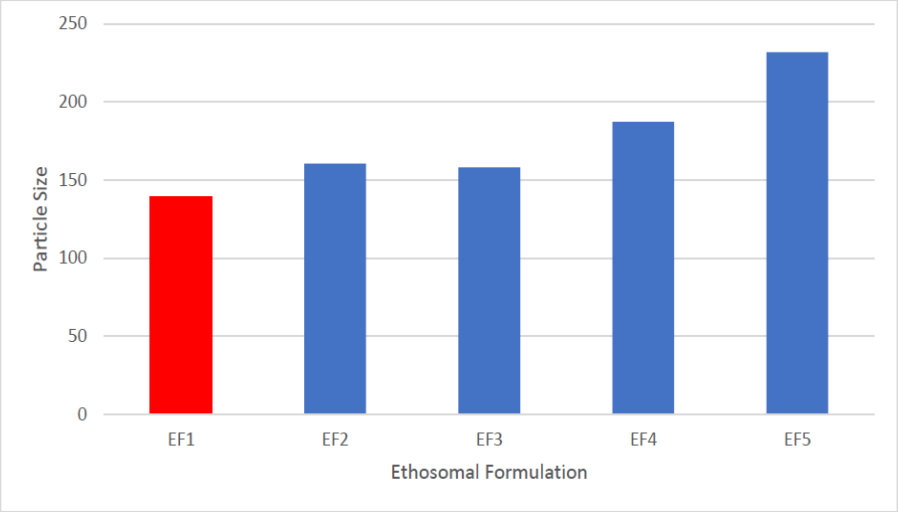

Fig. 01: Particle size of Ethosomal Formulations (EF1-EF5).

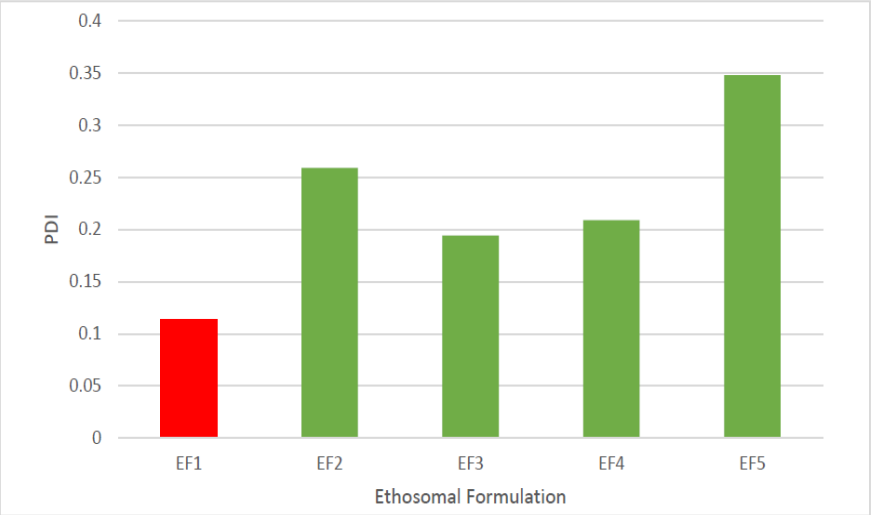

Fig. 02: PDI of Ethosomal Formulations (EF1-EF5).

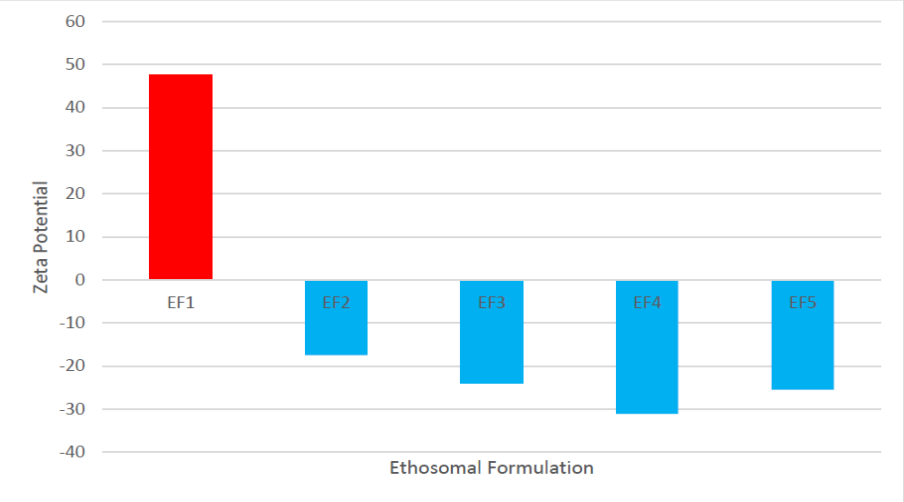

Fig. 03: Zeta Potential of Ethosomal Formulations (EF1-EF5).

Structural study of Kiwi and Rosemary extract loaded ethosomes by Transmission electron microscopy (TEM)

The surface structural analysis of the optimized EF1 formulation was conducted using TEM, as shown in Figure 04. TEM imaging provided detailed insights into both surface morphology and the spherical shape of the particles.

The images confirmed that the ethosomes were smooth, well-defined, and spherical, with no visible crystalline structure of the drug, indicating successful encapsulation (Bratu et al., 2011).

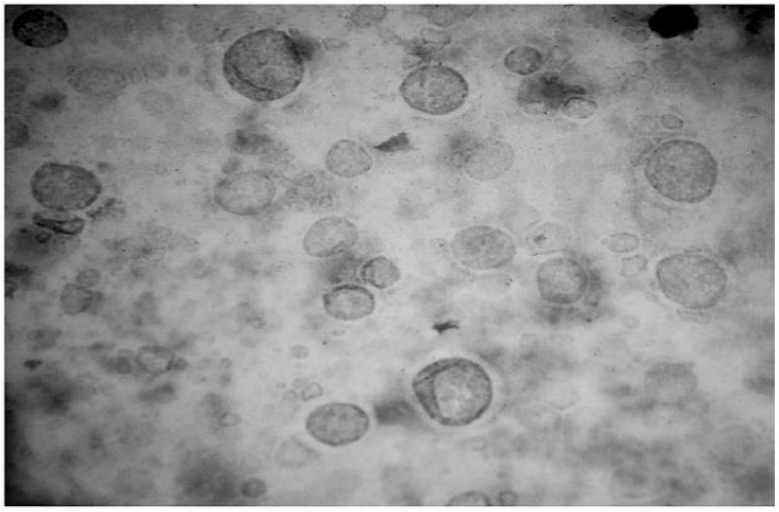

Fig. 04: Surface Morphology by TEM.

Morphology of Kiwi and Rosemary extract loaded ethosomes through Atomic force microscopy (AFM)

AFM images are presented in Figure 05. Unlike TEM images, which provide limited insight into the morphological characteristics, formulation behaviour, and swelling dynamics, AFM imaging offers a more detailed perspective.

Additionally, AFM analysis helps justify the external morphology of the molecule, particularly in terms of height, diameter, and surface area. The image clearly indicates that these parameters fall within an acceptable range. The diameter, height, and surface area of the ethosomal particles were determined to be 26.434 nm, 1.067 nm, and 547.814 nm^2, respectively (Mahant et al., 2018).

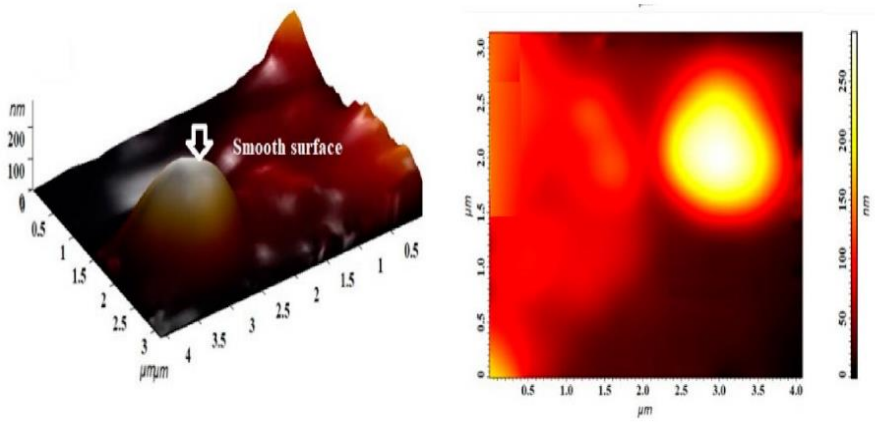

Fig. 05: Surface Morphology by AFM.

Calculation of Drug Entrapment efficiency, *EE (%)*

Entrapment efficiency is a key factor in evaluating the delivery potential of a formulation. The entrapment efficiency values for various ethosomal formulations containing Kiwi and Rosemary extracts are recorded in Table 03 or Table 04.

As illustrated in Figure 06, a graph was plotted for each formulation, revealing that EF1 had the highest entrapment efficiency at 89.43 ± 0.53%, while EF5 showed the lowest at 61.75 ± 0.63%. The entrapment efficiency for EF2, EF3, and EF4 was 71.48 ± 0.14%, 75.19 ± 0.29%, and 65.23 ± 1.63%, respectively.

Among the five tested ethosomal formulations, the ranking in terms of increasing entrapment efficiency was determined as EF1 > EF3 > EF2 > EF4 > EF5, consistent with previous findings (Ling et al., 2010; Shah et al., 2012).

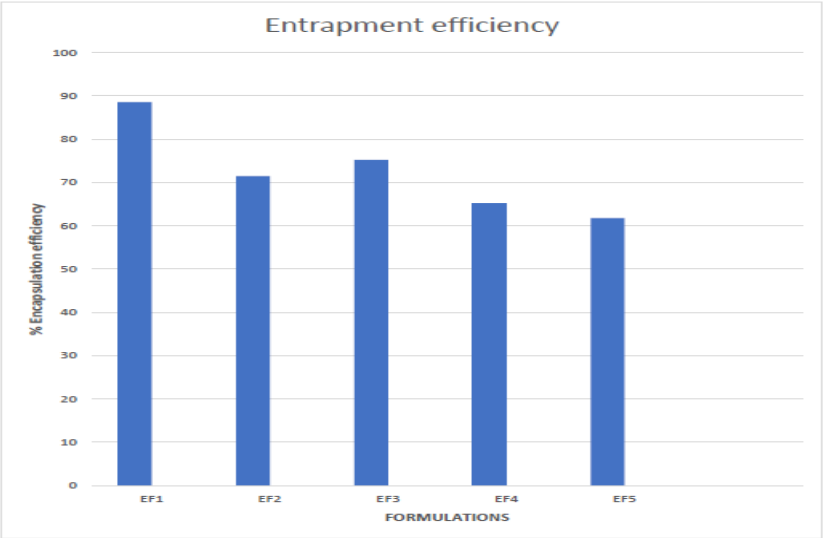

Fig. 06: EE (%) of ethosomal formation (EF1-EF5).

Determination pH, viscosity, Spreadability and Extrudability

The characteristics of all ethosomal formulations, including pH and viscosity, were found to range between 6.1 to 5.2 and 8528 ± 2.4 to 6845 ± 1.6 cps, respectively. A detailed summary of these findings is provided in Table 04.

Among the tested formulations, EF1 was identified as the optimized formulation, with a pH of 5.2 and a viscosity of 7232 ± 1.2 cps. Additionally, Table 03 highlights the impressive results obtained for the Spreadability and extrudability tests. EF1 demonstrated a Spreadability of 8.28 ± 0.6 cm and showed excellent extrudability (+++), aligning with previous research (Dave et al., 2017; Panigrahi et al., 2006).

Table 04: pH, Viscosity, Spreadability and Extrudability of different ethosomal preparations.

Characterization	EF1	EF2	EF3	EF4	EF5
pH	5.2	5.7	5.9	5.3	6.1
Viscosity	7232 ± 0.5	7063 ± 1.3	7521 ± 0.5	8528 ± 0.5	6845 ± 0.5
Spreadability	8.28 ± 0.6	6.20 ± 1.5	7.83 ± 0.3	8.05 ± 1.5	5.90 ± 0.8
Extrudability	+++	++	++	++	++

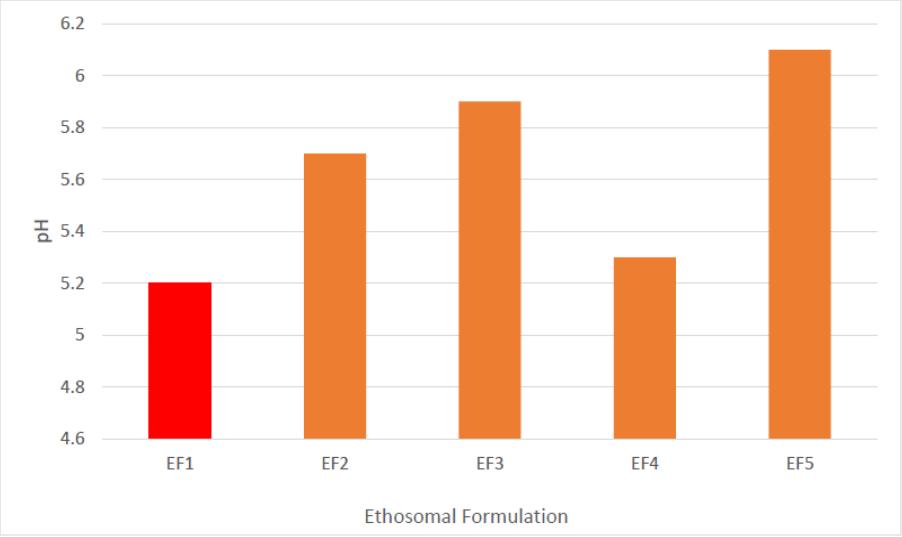

Fig. 07: pH value of ethosomal formation (EF1-EF5).

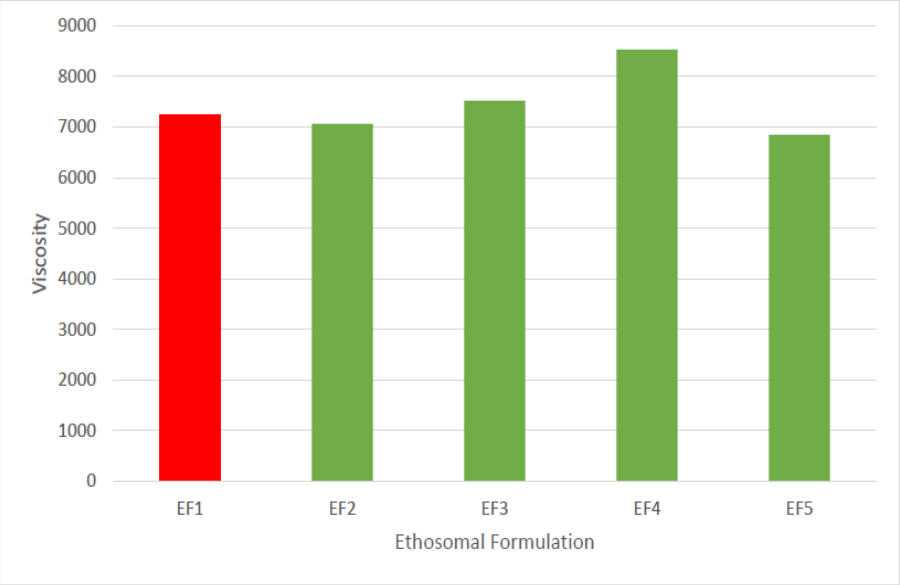

Fig. 08: Viscosity of ethosomal formation (EF1-EF5).

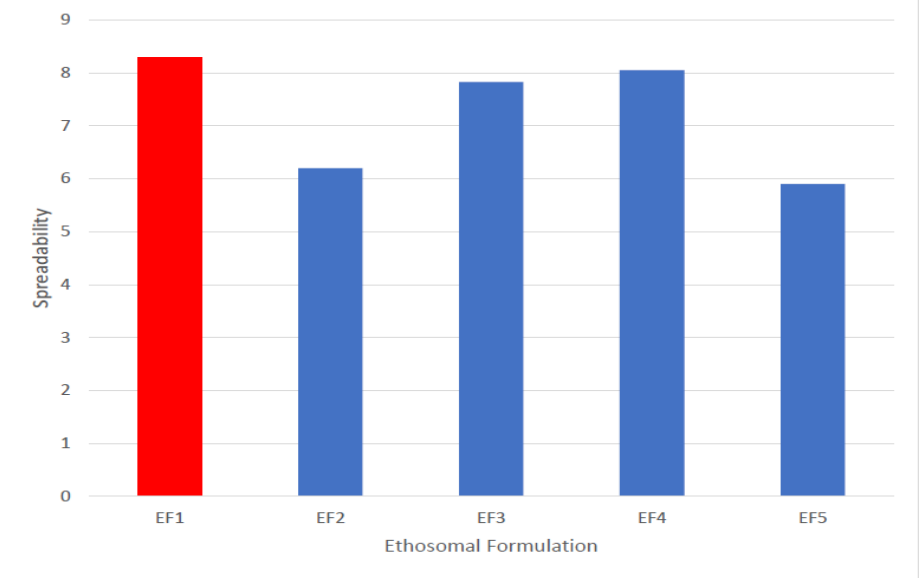

Fig. 09: Spreadability of ethosomal formation (EF1-EF5).

Determination of stability studies

A stability study was conducted to assess the ethosomal system's ability to adhere to medication over three months at different temperatures. The main issues with ethosomal formulation were drug agglomeration and drainage into/from the lipid bilayer. The optimized formulation EF1 was tested, and the results are presented in various tables. The lyophilized and un-lyophilized Kiwi and Rosemary extract loaded ethosome suspensions were stored at different temperatures. Sampling was conducted after 7, 15, 30, 60, and 90 days of hoarding. Following lyophilization, the non-aqueous sample was redistributed using DI water and further studies

were conducted to determine particle size, zeta-potential, PDI, and percent of entrapment efficiency. The course pf action was run three times to ensure precise findings, and the mean ±SD was used to publish the results.

Table 05: Stability study of optimized Kiwi and Rosemary extract loaded ethosomes particle suspension (EF1) at 4°C/60 ± 5 RH (n=3).

Time	Microscopic evaluation	% Encapsulation efficiency
Initial	Soft ball-shaped vesicle	93 ± 2.8
7	Soft ball-shaped vesicle	95 ± 1.3
15	Soft ball-shaped vesicle	93 ± 1.1
30	Soft ball-shaped vesicle	91 ± 2.6
60	Soft ball-shaped vesicle	87 ± 1.8
90	Rough ball-shaped vesicle	79 ± 2.2

Table 06: Stability study of optimized Kiwi and Rosemary extract loaded ethosomes particle suspension (EF1) at 25°C/60 ± 5 RH (n=3).

Time	Microscopic evaluation	% Encapsulation efficiency
Initial	Soft ball-shaped vesicle	95 ± 1.1
7	Soft ball-shaped vesicle	91 ± 1.6
15	Soft ball-shaped vesicle	87 ± 2.1
30	Soft ball-shaped vesicle	80 ± 2.3
60	Rough ball-shaped vesicle	66 ± 2.2
90	Clump	60 ± 1.3

Table 07: Stability study of optimized Kiwi and Rosemary extract loaded lyophilized ethosomes particle suspension (EF1) at 4°C/60 ± 5 RH (n=3).

Time	Microscopic evaluation	% Encapsulation efficiency
Initial	Soft ball-shaped vesicle	95 ± 1.1
7	Soft ball-shaped vesicle	95 ± 1.7
15	Soft ball-shaped vesicle	91 ± 1.8
30	Soft ball-shaped vesicle	87 ± 2.7
60	Rough ball-shaped vesicle	84 ± 1.4
90	Rough ball-shaped vesicle	80 ± 3.6

Table 08: Stability study of optimized Kiwi and Rosemary extract loaded lyophilized ethosomes particle suspension (EF1) at 25°C/60 ± 5 RH (n=3).

Time	Microscopic evaluation	% Encapsulation efficiency
Initial	Soft ball-shaped vesicle	93 ± 2.8
7	Soft ball-shaped vesicle	95 ± 1.3
15	Soft ball-shaped vesicle	93 ± 1.1
30	Rough ball-shaped vesicle	89 ± 2.5
60	Rough ball-shaped vesicle	83 ± 1.6
90	Clump	77 ± 2.0

***In vitro* study for skin permeation**

The study examined the release profile of Kiwi and Rosemary extract medication using a membrane filter. The ejection of the medication was significantly influenced by changes in ethanol and fat content. The cumulative drug release values for each formulation (EF1–EF5) were calculated using a linear line. Formulation EF1 displayed the highest drug release of 73.87 ± 0.63 due to significant drug trapping, while formulation EF3 had the lowest cumulative drug release. The lipid bilayer, which acts as a limiting barrier, sustained the drug release. The EF1 formulations showed quick drug release due to their low lipid concentration and presence of Glycerine. The content of lipid, Glycerine, and ethanol significantly impacted the drug's release from the formulation. After considering all parameters, it was determined that EF1 had an optimized formulation and was approved as a viable formulation for in-vivo research.

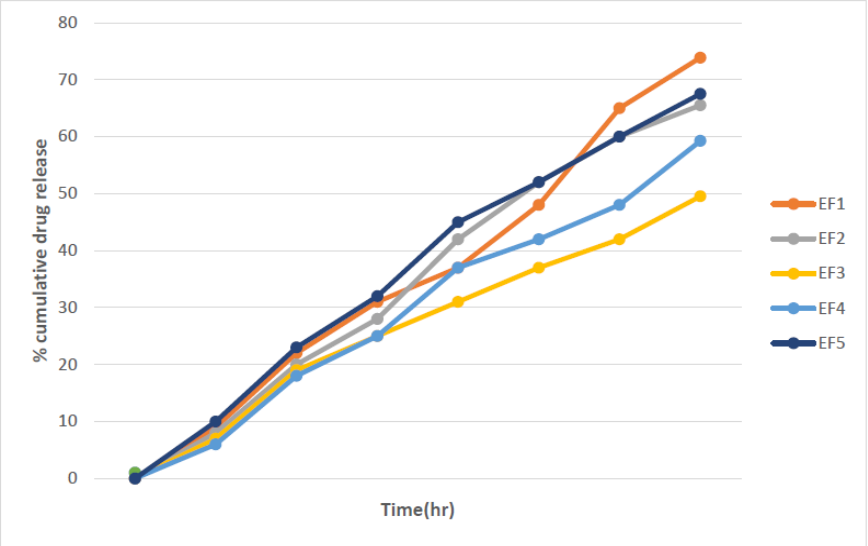

Fig. 10: In vitro skin permeation release profile for ethosomal formation EF1 to EF5 loaded with Kiwi and Rosemary extract for 24 h.

DISCUSSION

The development of ethosomal gel formulations for anti-aging applications represents a significant breakthrough in dermatological and cosmetic research. EF1, a formulation incorporating kiwi and rosemary extracts, soya-lecithin, ethanol, and propylene glycol, has demonstrated notable potential for topical skincare, particularly in addressing oxidative stress and enhancing skin rejuvenation. The choice of ingredients and their synergistic effects contribute to the formulation’s efficacy in combating the signs of aging. Kiwi extract, a rich source of vitamin C, polyphenols, and flavonoids, plays a vital role in neutralizing free

radicals, which are primary contributors to premature aging. Vitamin C is crucial for collagen synthesis, a key protein responsible for maintaining skin elasticity and firmness. By stimulating collagen production, kiwi extract aids in reducing the appearance of wrinkles and fine lines, making it an essential component of EF1. Additionally, the polyphenolic compounds found in kiwi provide protection against UV-induced oxidative damage, further preventing skin aging. Rosemary extract complements these effects with its high concentration of rosmarinic acid, carnosic acid, and essential oils, all of which exhibit strong antioxidant and anti-inflammatory properties. These bioactive compounds support skin repair, reduce inflammation, and protect against environmental stressors that accelerate aging. The presence of rosemary extract enhances microcirculation, promoting better oxygenation and nutrient delivery to skin cells, which results in healthier and more youthful-looking skin. The ethosomal system used in EF1 enhances the delivery and penetration of these active ingredients into deeper skin layers, maximizing their therapeutic benefits.

The physicochemical properties of EF1 play a crucial role in determining its effectiveness. The particle size and polydispersity index (PDI) are critical factors influencing the absorption and bioavailability of the formulation. EF1 demonstrated an optimal particle size, which facilitates better penetration through the skin barrier while maintaining stability. A smaller particle size ensures improved skin permeability, allowing for deeper absorption of the active compounds. Furthermore, the zeta potential of EF1 indicated good stability, preventing vesicle aggregation and maintaining the structural integrity of the ethosomal vesicles over time. The absence of xanthan gum in EF1 contributed to its enhanced spreadability and absorption into the skin, making it easier to apply and improving user experience. Additionally, the viscosity of the formulation was found to be within an acceptable range for topical applications, ensuring that the gel maintains its consistency without being too runny or too thick. The pH of EF1 was carefully maintained within the physiological range of human skin, preventing irritation and ensuring compatibility with different skin types. These parameters indicate that EF1 is a well- balanced formulation suitable for regular topical use.

The stability of ethosomal formulations is another key aspect of their effectiveness. Stability studies for EF1 revealed that the formulation retained its entrapment efficiency and physicochemical properties best when stored at 4°C/60 ± 5 RH. However, when stored at room temperature (25°C/60 ± 5 RH), a slight decrease in entrapment efficiency was observed, highlighting the need for controlled storage conditions to preserve the

integrity of active ingredients. The stability of EF1 is attributed to the optimized ethanol-to-lipid ratio, which prevents vesicle aggregation and degradation over time. Ethanol plays a crucial role in ethosome formation by fluidizing the lipid bilayer of the stratum corneum, enhancing skin penetration, and improving the bioavailability of kiwi and rosemary extracts. The choice of soya-lecithin further contributes to the structural integrity of the ethosomal vesicles while also acting as a skin-conditioning agent. Propylene glycol, another key component, functions as a humectant, ensuring adequate hydration and preventing the gel from drying out. The combination of these ingredients results in a stable, effective formulation with long-term usability.

The anti-aging efficacy of EF1 is driven by the synergistic action of kiwi and rosemary extracts. Kiwi extract, as a potent source of vitamin C, plays a critical role in collagen synthesis and skin repair. Collagen is essential for maintaining skin structure, and its degradation leads to sagging and the formation of wrinkles. By stimulating collagen production, EF1 helps restore skin firmness and elasticity. The flavonoids and polyphenols in kiwi further contribute to reducing oxidative stress, which is a major factor in premature aging. Rosemary extract enhances these effects by providing additional antioxidant protection and promoting blood circulation. Improved blood flow ensures that skin cells receive essential nutrients and oxygen, leading to a revitalized complexion. Additionally, rosemary's anti-inflammatory properties help soothe irritated skin, reducing redness and inflammation often associated with aging. The combination of these two extracts in an ethosomal system ensures their effective delivery into deeper skin layers, maximizing their therapeutic potential and making EF1 an advanced formulation for anti-aging applications.

Ethosomal drug delivery systems offer several advantages over conventional topical formulations. The presence of ethanol enhances permeability, allowing deeper penetration of active compounds into the skin. This is particularly beneficial for anti-aging applications, where active ingredients need to reach the dermis to stimulate collagen production and repair damaged skin cells. Compared to traditional creams and gels, ethosomal formulations like EF1 provide improved stability and controlled release of active ingredients, ensuring prolonged efficacy. The small vesicle size of ethosomes allows for better absorption, reducing the likelihood of surface residue and improving the overall user experience. Additionally, ethosomal gels are non-greasy, lightweight, and easily absorbed, making them suitable for daily skincare routines. The formulation's spreadability and extrudability were

found to be optimal, ensuring easy application and even distribution on the skin. The ability of ethosomes to enhance transdermal drug delivery makes them particularly suitable for anti-aging treatments, where deep skin penetration is necessary for effective results.

EF1 holds significant potential for commercial development as an anti-aging skincare product. Its natural composition, enhanced skin penetration, and antioxidant-rich profile make it a strong candidate for inclusion in cosmeceutical formulations targeting aging-related skin concerns. Further research is warranted to evaluate its long-term effects, including in vivo studies and clinical trials to assess its efficacy in reducing visible signs of aging. Additionally, exploring the incorporation of other bioactive compounds, such as hyaluronic acid, peptides, or plant- derived retinoids, could further enhance the formulation's effectiveness. Studies on consumer acceptability, sensory attributes, and dermatological safety will also be essential for product development and market positioning. With its promising attributes, EF1 represents a step forward in anti-aging skincare, offering a scientifically backed, naturally derived, and highly effective solution for maintaining youthful, healthy skin.

CONCLUSION

The EF1 formulation, incorporating kiwi and rosemary extracts in an ethosomal gel, demonstrated promising potential for anti-aging applications. The optimized combination of active ingredients and excipients contributed to its stability, skin permeability, and effectiveness.

Kiwi and rosemary extracts, rich in antioxidants, play a crucial role in combating oxidative stress, reducing fine lines, and enhancing skin elasticity. The presence of ethanol (4.5 ml) improved ethosome formation, ensuring better entrapment and penetration of active compounds into the skin. The balanced composition of soya-lecithin (1.5 g) and propylene glycol (3 g) contributed to the formulation's structural integrity and hydration properties, supporting prolonged skin nourishment.

Physicochemical evaluations confirmed that EF1 exhibited optimal particle size, polydispersity index (PDI), and zeta potential, ensuring formulation stability and effective skin absorption. The absence of xanthan gum in EF1 allowed for better Spreadability, making it ideal for topical application. Stability studies further indicated that EF1 maintained its properties best when stored at 4 °C/60 ± 5 RH.

In conclusion, the EF1 formulation displayed excellent physicochemical characteristics, effective antioxidant delivery, and suitable skin absorption, making it a strong candidate for anti-aging skincare applications. Further research and clinical evaluation are recommended to validate its long-term efficacy in reducing visible signs of aging.

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