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In Vitro Assessment of Herbal Topical Ethosomal Gel Formulation for the Treatment of *Acne vulgaris*

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Abstract

Anti-acne herbal formulations are utilized for the treatment of acne vulgaris with the additional benefit of not creating adverse effects, unlike synthetic drugs. Phytoconstituents present in methanolic extracts of *Glycyrrhiza glabra* have antibacterial and antioxidant properties. An approved UV spectrophotometer confirmed the presence of glycyrrhizin in these extracts. Extracts loaded with Carbopol® 940 were utilized for the preparation of herbal drug-loaded ethosomal gel formulations (EF1, EF2, and EF3) at various times and followed the assessment of ethosomal gel formulation. The evaluation of ethosomal gel formulation done by pH, viscosity, spreadability, texture analysis, differential scanning calorimetry, FT-IR spectral analysis, *in vitro* drug release study, and antioxidant activity against *Propionibacterium acnes*. Herbal ethosomal gel formulation demonstrated antibacterial potential and was additionally assessed for skin permeation by gamma scintigraphy utilizing hydrophilic radiotracer 99mTc-DTPA and lipophilic radiotracer 99mTc-MIBI. Significant permeation (1.3 µg/mg) was seen with a hydrophilic radiotracer named 99mTc-DTPA-PHF and this proposed that the formulation was equipped with sustained drug delivery for the treatment of moderate to an extreme type of acne.

Keywords: Acne, Antimicrobial screening, FT-IR, Gamma scintigraphy, Herbal ethosomal gel formulation, UV Spectrophotometer

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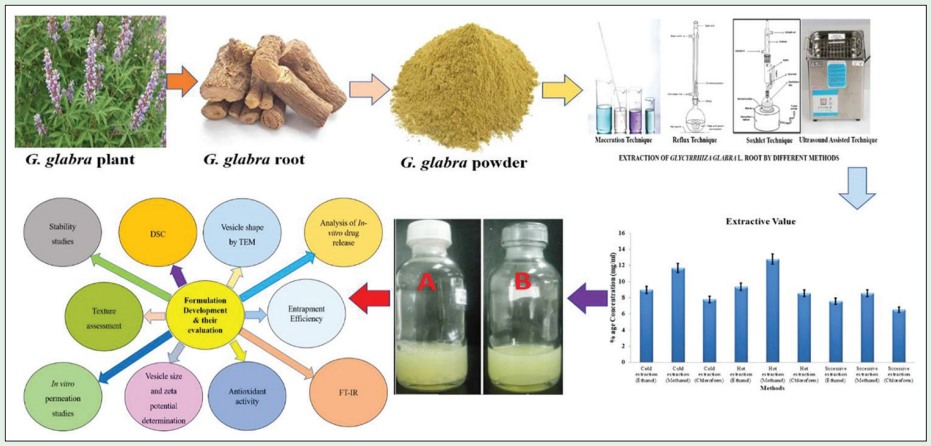
Structural abstract



Graphical representation of *Glycyrrhiza glabra* L. and their formulation evaluation

Introduction

Acne vulgaris is a cutaneous disorder of multifactorial beginning that shows in the pilosebaceous follicle. It is described by open and closed comedones and inflammatory lesions such as papules, pustules, and nodules.[1] Microorganisms such as *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermis* multiply quickly,[2] leading to the improvement of acne. In the clinical administration of acne vulgaris, a significant number of antibiotics and chemotherapeutic agents are accessible in the worldwide market as topical or systemic treatment modalities.[3] Skin treatment is liked as first-line treatment in gentle acne while, for moderate and extreme kinds of acne, fundamental treatment is needed in addition to topical therapy. Herbal therapies on the other hand are gaining attention in comparison to existing formulations which cause enormous side effects like skin dryness, rashes, wrinkling, erythema, pruritis, skin eruption, and development of resistance.[4] Several plants with antimicrobial, antibacterial, and antioxidant activity such as *Ocimum gratissimum*,[5] *Glycyrrhiza glabra*,[6] *Psidium guajava*,[7] *Garcinia mangostana*,[8] and *Humulus lupulus* L. were found to be effective[9] for prevention of acne vulgaris.

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The plant drug comprises the fresh root of *G. glabra*. The genus name *Glycyrrhiza* was gotten from the Greek glykys, for (sweet), and rhiza, for (root). The species name *Glabra* was gotten from the Latin glaber, which deduces (smooth) or (uncovered) and suggests the smooth husks. The plant principal names were: Arabic: Sus, Irik Sus, and rib el-sus; English: Licorice, licorice root, and liquorice; French: Réglisse; German: Lakritze and Süßholz; Hindi: Mulhatti, Jethimadh, and Mithilakdi; Italian: Liquirizia; and Portuguese: Alcaçuz.[10,11] Licorice is an herb utilized in nourishment and pharmaceutical for a huge number of years in the standard medication framework. It is herbaceous suffering, developing to 1 m in height, with pinnate leaves around 7–15 cm long, with 9–17 flyers. The blooms are 0.8–1.2 cm long, purple to pale whitish-blue, made in a free inflorescence. The natural product is an oval case, 2–3 cm long, containing a few seeds.[12]

An ideal formulation for acne should spread effectively and leave negligible residue or oiliness as it is intended for enormous for large hairy surfaces such as the chest and the back. Carbopol® 940 utilized for the formulation is an excellent viscosity builder even at low concentration and does not support microbial growth. In addition, it provides good plastic flow properties with significant yield value. Propylene glycol is a water-miscible cosolvent for Carbopol® 940 and acts as a preservative, humectant, plasticizer, or stabilizer in a variety of pharmaceutical formulations.[13] Its penetration enhancement ability has been attributed to the expanded transdermal flux of numerous drugs.[14]

In our past work, we had screened methanolic extracts for phytochemical, antibacterial, and antioxidant potential.[15,16] The fundamental goal of the current investigation is to plan and evaluate herbal ethosomal gel formulations loaded with methanolic extracts of *G. glabra* and to assess for *in vitro* permeation studies that were carried out using modified Franz diffusion cell to study. The release of drug from the prepared vesicles and drug released from a hydro-ethanolic solution. The results will be useful in designing specific, novel, and effective herbal anti-acne formulation for cosmetic and dermatological application to prevent the adverse effects of existing non-herbal formulations.

Materials and Methods

Authentication of Plant Material and Different Types of Extraction Method

The dried root of the plant material of *G. glabra* was bought from the nearby market of Khari Bawli, Delhi, India. The collected plant materials were taxonomically recognized and authenticated by Dr. H. B. Singh, Chief Scientist (RHMD) CSIR NISCAIR, New Delhi, India.

Preparation of *G. glabra* L. Roots Plant Material

The root was dried in a hot air oven at 50°C for 1 h to diminish moisture. The dried root was then made powder with the help of a grinder (Model-SUJATA) and passed through a sieve (20 meshes). The powdered sample was kept in an air-tight container and protected from sunlight and air until it is used.

Morphological Character

A detailed study of the morphological characters can help separate them. The macroscopy of a drug includes its visual appearance to the naked eye. It depends generally on the plant from which the drug is obtained. Every specific morphological group, a particular systemic examination, can be carried out. Size, color, odor, and taste are significant parts of the morphology of a particular drug.

Extraction of *G. glabra* L. Root by Different Methods

Dry powdered of the plant *G. glabra* L. root was extracted with different methods of extraction. Extraction and comparison of extractive techniques were performed for glycyrrhizin content in Table 1 and Figure 1.

- Maceration technique
- Soxhlet apparatus
- Reflux technique
- Ultrasonic-assisted extraction.

Extraction by maceration technique

About 2–4 g of dried root powder of *G. glabra* L. were extracted using different organic solvents such as methanol, petroleum ether, chloroform, and acetone, (drug:solvent = 1:25) by maceration method for 7 days in each solvent. The extracts were evaporated to dryness using a rotary vacuum evaporator (HAHN SHIN, HS-2005 V-N) at 40°C under an inert atmosphere to obtain amber to black colored aqueous mass. The extract obtained was reconstituted with methanol and evaporated to dryness to get amber to black-colored extract which was weighed and was subjected to further quantification.

Extraction by reflux technique

The coarse powder of the dried root of *G. glabra* L. was extracted with different solvents such as methanol, petroleum ether, chloroform, and acetone (drug: solvent ratio is 1:25) by hot solvent extraction in a reflux condenser assembly for 5 h each. The extracts obtained were concentrated under reduced pressure in a rotary vacuum evaporator (HAHN SHIN, HS-2005 V-N) at 40°C to obtain black-colored extract aqueous mass.

Extraction by Soxhlet apparatus

The coarse powder of the dried root of *G. glabra* L. was extracted with various solvents such as methanol, petroleum ether, chloroform, and acetone (drug: solvent = 1:25) for 5 h each using Soxhlet apparatus. The different extracts were concentrated under reduced pressure in a rotary vacuum evaporator (HAHN SHIN, HS-2005 V-N) at 40°C to obtain a black-amber-colored aqueous mass.

Extraction by ultrasound-assisted extraction technique

The coarse powder of the dried root of *G. glabra* L. was extracted with various solvents such as methanol, petroleum ether, chloroform, and acetone (drug: solvent = 1:25) for 30 min at 50°C. The different extracts were concentrated under reduced pressure

Table 1: Percentage yield of *Glycyrrhiza glabra* by different extraction methods

S. No.	*Plant name*	*Extraction method*	*Value (%w/w)*
1.	*Glycyrrhiza glabra* Root	Maceration	10.562
2.		Soxhlet apparatus	7.893
3.		Reflux	8.368
4.		Ultrasonic-assisted extraction	14.378

in a rotary vacuum evaporator (HAHN SHIN, HS-2005 V-N) at 40°C to obtain a black-amber-colored aqueous mass. The extract obtained was reconstituted with methanol and evaporated to dryness to get a black-amber-colored extract which was weighed and was subjected to further quantification Figures 3 and 4.

Pre-formulation Studies

Physical properties

Glycyrrhizin sample was observed for physical appearance, pH, odor, melting point, etc., as shown in Table 2 and Figures 5-7.

Identification Tests

UV analysis

One milligram of glycyrrhizin was dissolved in 10 ml methanol. Diluted 5 ml of the above solution with methanol to 100 ml. Examined between 200 nm and 400 nm.

Analytical Methodology for Formulation

Preparation of working solution

Various working solutions were prepared freshly when needed according to I.P. 1996 and USP 2000. Phosphate buffers (pH 7.4) were mainly used for release studies and were prepared accordingly.

Determination of λ_{max} by U.V. spectrum

The UV scan of glycyrrhizin was taken and λ_{max} was observed in different media such as –

- Methanol
- Phosphate buffer (pH 7.4).

Table 2: Physical properties of glycyrrhizin

S. No.	*Analysis items*	*Specifications*	*Results*	*Test methods*
1.	Identification	Positive	Confirms	UV
2.	Physical appearance	Crystalline powder	Confirms	Visual
3.	Color	White	Confirms	Visual
4.	Odor	Characteristic	Confirms	Organoleptic test
5.	Melting point	220°C	222°C	Capillary method
6.	pH 1% solution	5.5	5.04	Digital pH meter

Table 3: Absorbance at 254 nm in methanol by U.V. spectrophotometer

S. No.	*Concentration (µg/ml)*	*Absorbance at 260 nm*
1.	4	0.126
2.	8	0.239
3.	12	0.378
4.	16	0.672
5.	20	0.862
6.	24	0.98

Table 4: Absorbance at 256 in phosphate buffer by U.V. spectrophotometer

S. No.	*Concentration (µg/ml)*	*Absorbance at 256 nm*
1	4	0.364
2	8	0.426
3	12	0.539
4	16	0.682
5	20	0.798
6	24	0.932

The prepared solutions were scanned for UV absorption in the wavelength range 200–400.

Preparation of calibration curves

In methanol

One milligram of glycyrrhizin was dissolved in 10 ml of methanol to form a stock solution of 100 µg/ml. For UV analysis, a series of glycyrrhizin solutions (2–20 µg/ml) was prepared from the stock solution by dilution with methanol. A calibration curve was plotted between concentration (µg/ml) and absorbance.

In phosphate buffer (pH 7.4)

One milligram of glycyrrhizin was dissolved in 10 ml of phosphate buffer (pH 7.4) to form a stock solution of 100 µg/ml. For UV analysis, a series of glycyrrhizin solutions (2–20 µg/ml) was prepared from stock solutions by dilution with phosphate buffer (7.4 pH). A calibration curve was plotted between concentration (µg/ml) and absorbance.

Preparation of herbal topical ethosomal gel formulation

The composition of the placebo ethosomal formulation is presented in Table 5. Various concentrations of phospholipids, ethanol, phosphate buffer, etc., were tried while changing the RPM of rotation of the round bottom flask (RBF) and temperature of the water bath in which the RBF containing organic phase was immersed to find out the best and homogenous formulation. It was observed that with an RPM of 240 and a temperature of 50°C, a very thin, uniform, and desirable layer around RBF was obtained. A total of six placebo formulations with different combinations were chosen for the preparation of drug-loaded formulation. The interpretation was done based on the physical stability of formulations and particle size. The best, optimized placebo formulations with different combinations were chosen for the preparation of drug-loaded formulations. Figure 2 shows a graphical representation of formulation development and its evaluation parameters.

Preparation of drug-loaded ethosomes

Once the combinations of various segments in ethosomal formulation were set according to the placebo ethosomal formulation, drug-loaded ethosomes were prepared using these optimum ranges. Phospholipid 100S concentrations for the preparation of drug-loaded ethosomes were increased (i.e. for encapsulation of larger amounts of the drug, the phospholipid concentration needed to be increased too; concentration was increased × 10). Three formulations were initially prepared with different concentrations of drugs (1.0%, 1.5%, and 2.0%) and were then further subjected for interpretation to select the best combination. The combinations of the drug-loaded ethosomal formulations are presented in Table 6 and Figure 10.

Characterization of Herbal Ethosomal Gel Formulations

Determination of pH and viscosity

Herbal gel formulations were diluted with distilled water (1:10) and pH was estimated utilizing Lab India pH meter (model Pico). The viscosity of herbal gel formulations was determined using Brookfield Viscometer (Brookfield Engineering Laboratories,

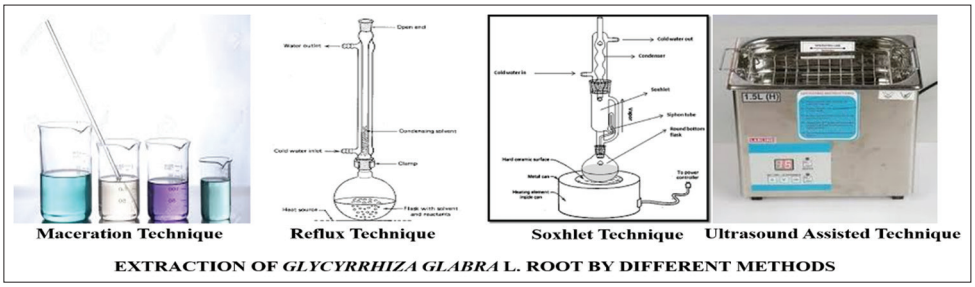

Figure 1: Different extraction methods of *Glycyrrhiza glabra* root

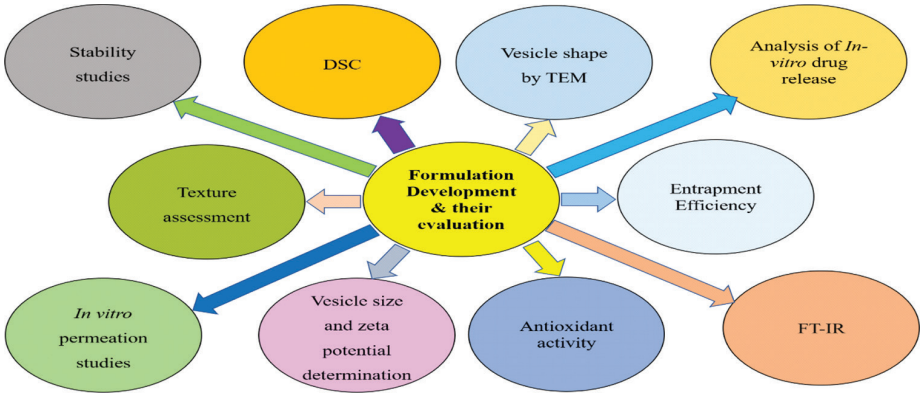

Figure 2: Graphical representation of formulation development and their evaluation parameters

Table 5: Composition of placebo ethosomal formulations

Formulation code	*Phospholipid 100S (% w/v)*	*Chloroform:methanol (v/v)*	*Ethanol:IPB (v/v)*	*Interpretation*
EP 1	15	2:1	25:75	++-
EP 2	30	3:2	35:65	+++
EP 3	20	2:1	30:70	+++
EP 4	15	3:2	25:75	++-
EP 5	25	1:2	25:75	+++
EP 6	20	2:1	30:70	++-

Interpretation* - Interpretation was done using three parameters, (a) vesicle size, (b) physical appearance, and (c) stability of ethosomes. Therefore, + signifies indicates that whether these parameters were in range or not, in the above-stated order.

Table 6: Composition of drug-loaded ethosomal formulations

Formulation code	*Phospholipid 100S (mg) (% w/v)*	*Ethanol:buffer (% v/v)*	*Drug concentration (%w/v)*	*Probe sonication time (min)*
EF-1	25	25:75	2.0	6 min
EF-2	30	35:65	1.0	4 min
EF-3	20	30:70	1.5	6 min

USA) with spindle # C 50-1 having a speed of 50 rpm. All the measurements were done in triplicate at room temperature.[17]

Determination of spreadability

Spreadability (g.cm/sec) is communicated as far as the time taken in seconds by two slides to sneak off from the gel placed between them, under a specific load.[18] The standardized weight tied on the upper plate was 20 g and the length of the glass slide was 6 cm. The lesser time taken for the partition of the two slides, the better is the spreadability. The spreadability was determined by utilizing the following formula:

Spreadability = (Weight Length)/Time

Texture assessment

In this investigation, a texture analyzer (Farnell QTS25 CNS, UK) furnished with computer software was utilized to survey the texture properties of the pastille samples. Pastille samples were rotated in two reciprocating cycles using round cylinder probes with a diameter of 5 cm, probe speed of 1 mm/s, trigger force of 0.1 N, and up to 40% of the initial sample height. Samples were compressed and then depressed. Texture properties from the force-deformation curve included stiffness, the maximum force required to compress the substance (equivalent to the maximum force at the compression stage); cohesiveness, and texture consistency. Cohesion connects the components of the matter. Tissues that are interconnected and their components are fully interconnected. It is the force that connects molecules in the substance; chewiness, gumminess of the texture resulted from hardness, and cohesiveness. It is necessary to chew and paste samples to swallow[19] and gumminess, a combination of hardness and cohesion, including the force needed to eat semi-solid food.

Vesicle size and zeta potential determination

The mean droplet size distribution, as well as the zeta potential of MEs, was performed at ambient temperature by photon correlation spectroscopy using Malvern Zetasizer Nano ZS, Malvern Instrument Ltd., Worcestershire, UK.

Droplet shape and morphology

The shape and morphology of microemulsion beads were analyzed by transmission electron microscopy (TEM) utilizing JEOL, JEM-1230 Transmission Electron Microscope, Tokyo, Japan. The microemulsion beads were checked after negative staining with 2% phosphotungstic acid.

DPPH radical scavenging activity (Antioxidant activity)

Radical scavenging activity of test samples (ethanolic solutions of rutin as a standard antioxidant, liquorice extract, and optimum ME) was tested at the concentration range of 20–640 mg/ml. A volume of 4 ml DPPH solution in absolute ethanol, 20 mg/l[20] was mixed with 1 ml of test sample; the absorbance was read immediately for the control (without test samples) (Ac) and after 30 min incubation in the dark at room temperature, for the samples (As), at 517 nm.[21] The experiments were conducted in triplicate. The percentage DPPH scavenged by the test samples was calculated as % scavenging effect = (Ac–As)/Ac × 100. The percentage scavenging effect was plotted versus samples' concentration. The scavenging concentration of each sample at 50% (SC50) was used to compare free radical scavenging activity.

Entrapment efficiency (EE)[22]

EE was estimated utilizing an 1800 UV–visible spectrophotometer (Shimadzu). A known measure of the prepared complex was ultracentrifuged at 5000 rpm for 15 min in methanol. The measure of phenolic compounds in the supernatant was then analyzed by UV–visible spectrophotometer at λ max 765 nm; the concentration of phenolic compounds was measured. All measurements were performed in triplicate. The entrapment efficiency results are shown in Table 9.

The EE was calculated using the following equation:

EE (%) = T–S/T × 100

Where

T: Total amount of phenolic compounds present in a quantity of phytophospholipid complex taken

S: Amount of phenolic compound in the supernatant

T–S: Measure of phenolic compounds entrapped

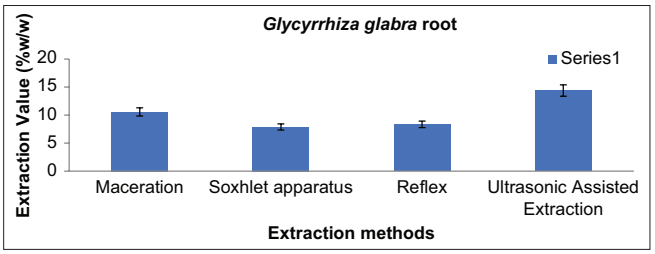

Figure 3: Extractive value of *Glycyrrhiza glabra* by different methods

Differential scanning calorimetry (DSC)

Orange peel extract, liquorice extract, phospholipid, physical mixture of extracts and phospholipid, and phytophospholipid complex were set in the aluminum crease cell and warmed at the speed of 10°C min^{-1} from 0 to 300°C in the atmosphere of nitrogen. The peak transition onset temperatures were recorded through an analyzer.

Vesicle shape by TEM

The shape of the liposomal vesicles was checked by TEM. TEM was done by negative staining technique using Formvar 100# copper grids. The liposomal suspension was diluted 1:100 with filtered distilled water. This diluted sample was mixed with 1% uranyl acetate stain in a 1:1 ratio. A drop of this was placed on the grids and allowed to stand. After 2–3 min, the excess sample was removed and the sample loaded grid was allowed to dry at room temperature for 10–12 h. The LDPI was reconstituted in filtered distilled water and the vesicle shape was checked similarly as for liposome suspension. Imaging was done using Carl Zeiss Libra® 120 Energy Filter Transmission Electron Microscope (Carl Zeiss, Oberkochen, Germany).

Stability studies

The microemulsion systems containing 1.5% liquorice extract were stored, in duplicate, at room temperature, and at 40°C, in an incubator (Shel Lab, model 1545, Sheldon Manufacturing Inc., Cornelius, OR) for 3 months. At 0, 15, 30, 45, 60, 75, and 90 days, the samples were examined for phase separation by visual inspection, and both the flavonoid and polyphenol contents in the formulations were determined.

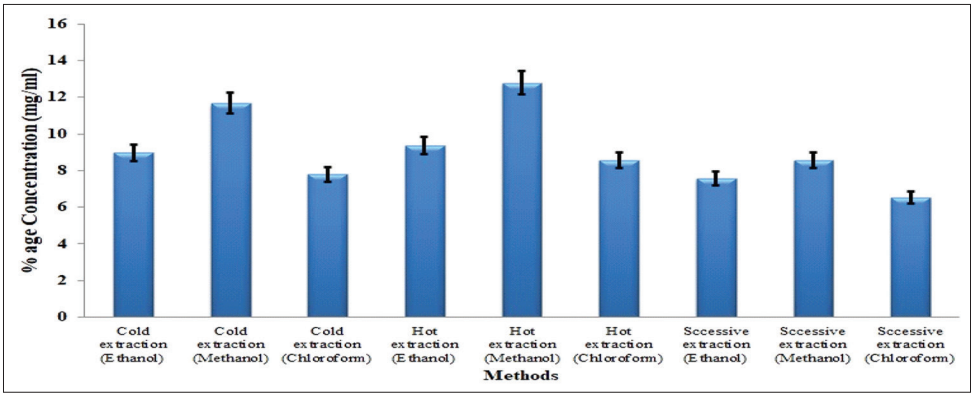

Figure 4: Extractive value of *Glycyrrhiza glabra* by different solvent methods

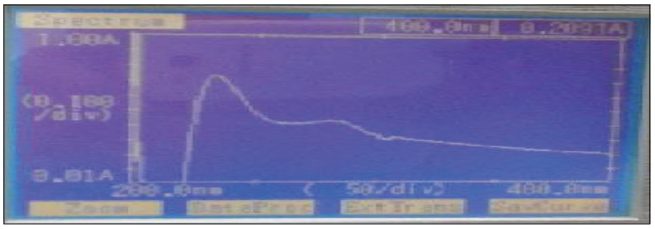

Figure 5: UV spectra of glycyrrhizin in methanol and showed an absorption maximum at 254 nm

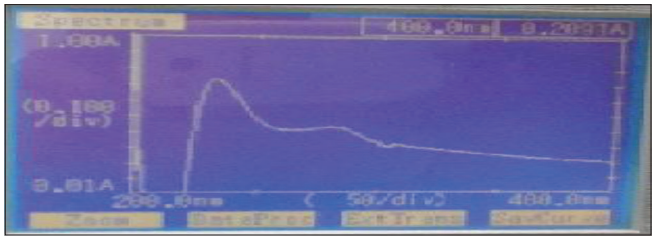

Figure 6: UV spectra of glycyrrhizin in methanol

In vitro permeation studies

In vitro skin permeation of MAG-loaded nanoemulsion formulations was studied using a locally manufactured Franz diffusion cell with an effective permeation area and receptor cell volume of 2.5 cm^2 and 10 ml, respectively. The temperature was maintained at 37 ± 0.5°C. The receptor compartment contained 10 ml phosphate buffer (pH 7.4) and was constantly stirred by a magnetic stirrer (Expo India Ltd., Mumbai, India) at 600 rpm. Dermatome (500 µm thickness) human cadaver skin from abdominal areas was obtained from the Chhattisgarh Institute of Medical Sciences (Bilaspur, India) and stored in a deep freezer at −20°C until further use. The skin was then carefully checked through a magnifying glass to ensure that samples were free from any surface irregularity, such as tiny holes or crevices in the portion that was to be used for transdermal permeation studies.[23] After verification, the skin was mounted onto a receptor compartment with the stratum corneum side facing the donor compartment. The donor compartment contained 1 g of the sample and sealed it with paraffin film to provide occlusive conditions. Samples were withdrawn through the sampling port of the diffusion cell at predetermined time intervals for 24 h, then analyzed for drug content using a UV Spectrophotometer at 251 nm (Shimadzu 1700, Japan). The receptor phase was immediately replenished with an equal volume of fresh diffusion buffer. The formulation NE2 provided the highest release, compared to the other nanoemulsion formulations. The formulation NE2 was also converted into nanoemulsion gel formulations, by adding 1% wt/wt Carbopol 940. This was coded as nanoemulsion gel (NG2), with the Carbopol 940 used as a viscosity modifier and gelling agent. The skin permeation profile of the optimized nanoemulsion formulation was compared to that of the NG2 and conventional gel, using Dunnett's test or one-way analysis of variance. All experiments were performed in triplicate. Sink conditions were maintained throughout the experiment. The drug release results are shown graphically in Tables 10-12 also Figures 18 and 19.

Fourier transform infrared spectrophotometry (FT-IR)

FT-IR Spectrometer, BRUKER IFS-55, Switzerland, was employed to examine the collaboration between extracts and phospholipids and to ascertain the structure and chemical stability of phytophospholipid complex, phospholipid, and extracts. The IR spectra of extracts, phospholipids, their complex, and physical mixture were obtained by the potassium bromide (KBr) method. KBr pellets were prepared by gently mixing the 1−mg sample with

Table 7: Physicochemical characterization of optimized ethosomal gel formulation

Color	*Appearance*	*Washability*	*Phase separation*	*Odor*
Light yellowish	Translucent	Washable	No	No
Homogeneity	*Drug content (%)*	*pH*	*Spreadability (g.cm/sec)*	*Extrudability (gm)*
Good/ excellent	90.78±2.69	6.5±0.58	15.22±3.62	6.12±1.32
Cohesiveness (gm)	*Consistency (gm. sec)*	*Firmness (gm)*	*Index of viscosity*	
−165.80	298.27	203.09	−193.45	

Table 8: Particle size comparison of drug-loaded ethosomes

Formulation code	*Z-average (d.nm)*	*PDI*
EF-1	130.12	0.136
EF-2	110.45	0.201
EF-3	253.46	0.098

PDI: Polydispersity index

Table 9: Entrapment efficiency of drug-loaded ethosomal formulation

Formulation code	*Drug (%w/v)*	*Entrapment efficiency (% E.E)*
EF-1	2	78.71
EF-2	1	84.82
EF-3	1.5	68.45

Table 10: Stability studies of ethosomes vesicles

Formulation code	*Time after preparation (days)*	*Average vesicle size (nm) (Mean ± S.D)*		
		25°C±2°C	*37°C±2°C*	*45°C±2°C*
EF-1, EF-2, and EF-3	01	130.12±3.62	110.45±4.12	253.46±2.89
	14	131.02±3.81	113.84±4.55	256.33±4.12
	30	132.42±3.93	114.93±4.67	257.48±4.76
	75	132.23±4.87	113.35±4.52	159.44±4.07
	90	133.24±4.82	114.74±4.46	158.32±4.80

Table 11: *In vitro* skin permeation of drug from hydroethanolic solution (control, C-1) in phosphate buffer (pH 7.4) using modified Franz diffusion cell (Group-1)

Time (h)	*Absorbance (nm)*	*Concentration (µg/ml)*	*The cumulative amount of drug permeated (µg/cm^2)*
0	0	0	0
0.5	0.038	7.89	54.5
1	0.046	9.9	68.39
2	0.081	20.22	139.68
4	0.146	38.96	269.14
6	0.169	46.23	319.36
8	0.193	52.52	362.822
12	0.211	59.32	409.79
Formulation code	*Flux (µg/cm^2/h)*	*Drug retaining in the skin (µg/mg)*	
Control (C-1)	35.118	0.82	

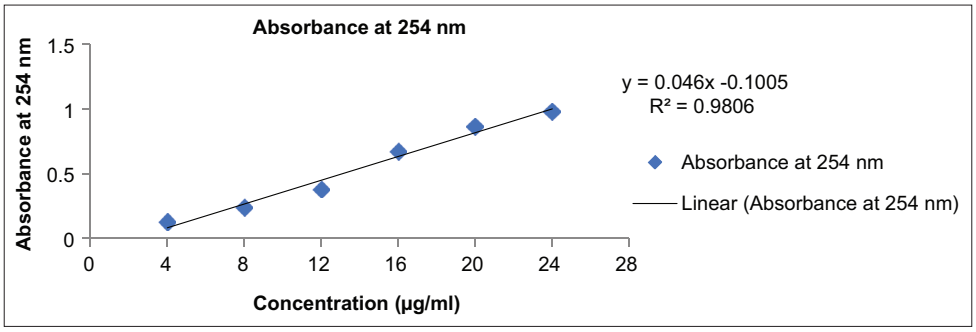

Figure 7: Calibration curve of *Glycyrrhiza glabra* in methanol

Table 12: *In vitro* skin permeation of drug from optimized ethosomal formulation (EF-2) in phosphate buffer (pH 7.4) using modified Franz diffusion cell

Time (h)	*Absorbance (nm)*	*Concentration (µg/ml)*	*The cumulative amount of drug permeated (µg/cm²)*
0	0	0	0
0.5	0.054	12.45	86
1	0.094	22.79	156.74
2	0.189	52.59	363.3
4	0.337	95.09	656.9
6	0.42	119.62	826.36
8	0.457	129.21	892.61
12	0.514	146.97	1015.3
Formulation code	*Flux (µg/cm²/h)*	*Drug retaining in the skin (µg/mg)*	
EF-1	88.808	1.3	

Table 13: Summary of peaks assigned to different groups in FT-IR spectra of glycyrrhizin and glycyrrhizin-loaded formulation

Chemical groups	*Glycyrrhizin (absorbance)*	*Glycyrrhizin-loaded formulation (absorbance)*
-OH stretching	3454.50 cm^{-1}	3421.72 cm^{-1}
C=H stretching of alkane	2929.87 cm^{-1} and 2875.87 cm^{-1}	2924.09 cm^{-1}, 2856.58 cm^{-1}
-C=O stretching of ketone	1726.29 cm^{-1}	1737.86 cm^{-1}
C=C stretching vibration	1651.06 cm^{-1}	1641.42 cm^{-1}
C=OH stretching	1080.14 cm^{-1}	1107.14 cm^{-1}

FT-IR: Fourier-transform infrared

100 mg KBr. Spectral scanning was done in the range between 4000 and 400 cm^{-1}.

Analysis of in vitro drug release kinetics

The mechanism of release was determined by fitting the release data to the various kinetic equations such as first-order,[24] zero-order, Higuchi,[25] and Korsmeyer–Peppas[26,27] and the R^2 values of the release profile corresponding to each model were found. The results are shown in Figure 21.

Results and Discussion

Percentage Yield by Different Extraction Methods

Dry powdered of the plant Glycyrrhiza glabra L. root was extracted with different methods of extraction. Extraction and comparison of extractive values mentioned in Table 1.

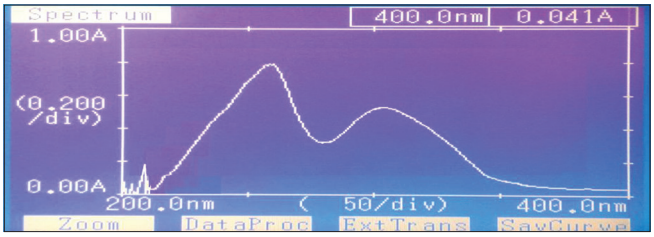

Figure 8: UV spectra of glycyrrhizin in phosphate buffer (pH 7.4)

Pre-formulation Studies

Physical properties

Glycyrrhizin sample was observed for physical appearance, pH, odor, melting point, etc.

Identification Tests

UV analysis

One milligram of glycyrrhizin was dissolved in 10 ml methanol. Diluted 5 ml of the above solution with methanol to 100 ml. Examined between 200 nm and 400 nm.

Preparation of Calibration Curves

In methanol

The calibration curve of glycyrrhizin was prepared in methanol at λ_{max} 254 nm for U.V. analysis. A positive correlation exists between the two variables, that is, concentration and absorbance for glycyrrhizin as revealed by the correlation coefficient ($R^2 = 0.9806$). The concentration and the corresponding absorbance are listed in Table 3.

In phosphate buffer (pH 7.4)

The calibration curve of glycyrrhizin was prepared in phosphate buffer (pH 7.4) at λ_{max} 256 nm for U.V. analysis. A positive correlation exists between the two variables, that is, concentration and absorbance for glycyrrhizin as revealed by the correlation coefficient ($R^2 = 0.9897$). The concentration and the corresponding absorbance are listed in Table 4 and Figure 8.

Preparation of Herbal Topical Ethosomal Gel Formulation

Six such formulations were prepared according to the above-stated combinations and the best ones were chosen for further

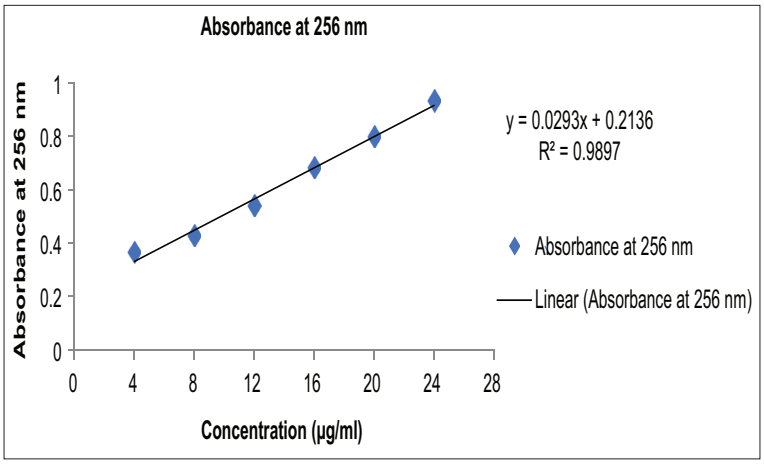

Figure 9: Calibration curve of glycyrrhizin in phosphate buffer (pH 7.4)

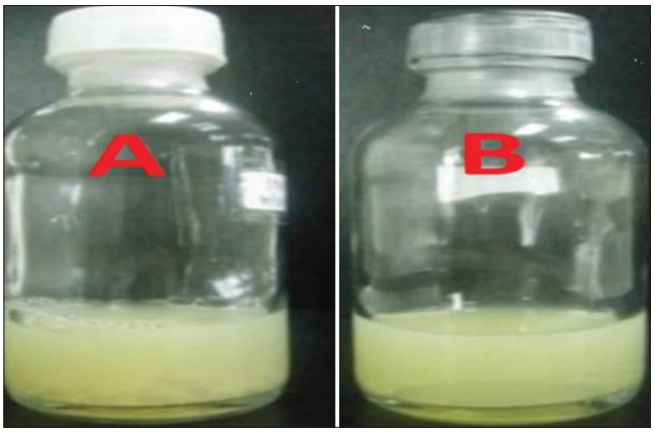

Figure 10: Drug-loaded ethosomal formulations

development of drug-loaded ethosomes. It was observed that chloroform: methanol ratio was the best to form a thin film in the RBF; therefore, this ratio was used in all further developments. It was also observed that 25 mg and 30 mg phospholipid were optimum for ethosomes preparation. The optimum concentration of the hydrating solution used was (a) 25:75 and (b) 35:65.

Preparation of Drug Loaded Ethosomes

Various ethosomal formulations have been reported for topical drug delivery for multiple diseases. Different formulations of herbal drug loaded ethosomes were formulated by varying the concentration of ethanol and phospholipid mentioned in Table 6. 3 formulations were initially prepared with different concentrations of drugs (1.0%, 1.5%, and 2.0%) and were then further subjected for interpretation to select the best combination. The combinations of the drug-loaded ethosomal formulations are presented below in Table 6 and Figure 10.

Characterization of Herbal Ethosomal Gel Formulations

Determination of pH and viscosity

Herbal gel formulations pH and viscosity also mentioned in Table 7.

Determination of spread-ability

The developed gel formulation was evaluated for different physical parameters, and outcomes are shown in Table. The developed gel formulation exhibited a pleasant and homogeneous appearance, without the presence of any rough particles. The pH of the developed dermal gel was recorded at 6.5 ± 0.58, which is compatible with skin and regarded as safe to avert the possibility of any skin irritation upon application. The spreadability of gel is another important parameter in terms of patient compliance and uniform distribution of formulation to the affected site. The developed gel formulation showed a spreadability and extrudability of 15.22 ± 3.62 g.cm/s and 6.12 ± 1.32 g, respectively.

Texture analysis

Texture analysis was performed for optimized formulation and Carbopol 940 gel (as control formulation), and different factors such as cohesiveness, consistency, firmness, and index of viscosity were assessed. The results of the texture analysis of the Carbopol gel and optimized formulation were obtained from software and have been reported in table, and texture image represented in figure. It was found that the optimized formulation showed cohesiveness (−165.80), consistency (298.27), firmness (203.09), and index of viscosity (−193.45), respectively.

Vesicle size and size distribution analysis

The average size of the ethosomes vesicles as measured by Malvern Instrument was found out to be in the range of 90–150 nm formulations EF-1 to EF-3. The polydispersity index showed that all the ethosomes had a narrow size distribution. The results are presented in Table 8 and Figures 11-13.

Antioxidant activity

The antioxidant activity of hydroalcoholic extract of *G. glabra* was determined by the DPPH assay in which the utilization of a stable free radical (DPPH) was measured. When a solution of DPPH (used as an indicator) is admixed with an antioxidant compound that

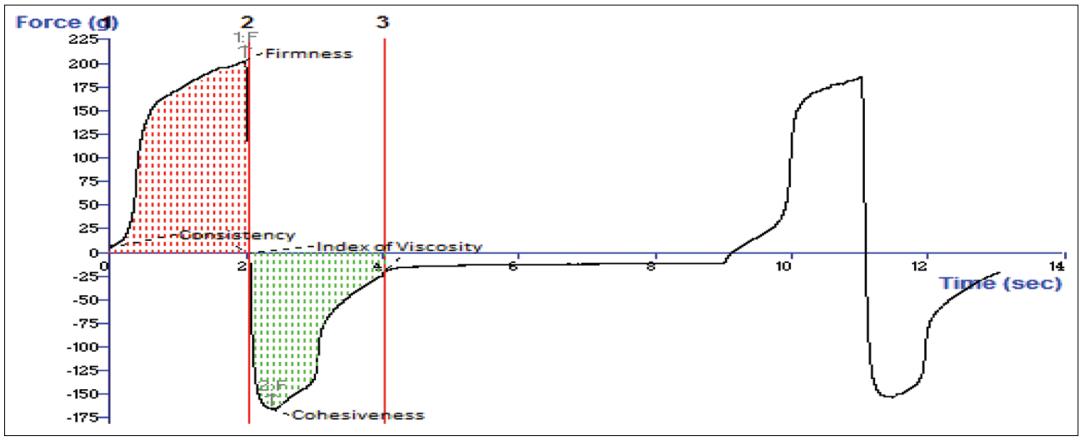

Figure 11: Texture analysis of the drug-loaded optimized ethosomal formulation gel

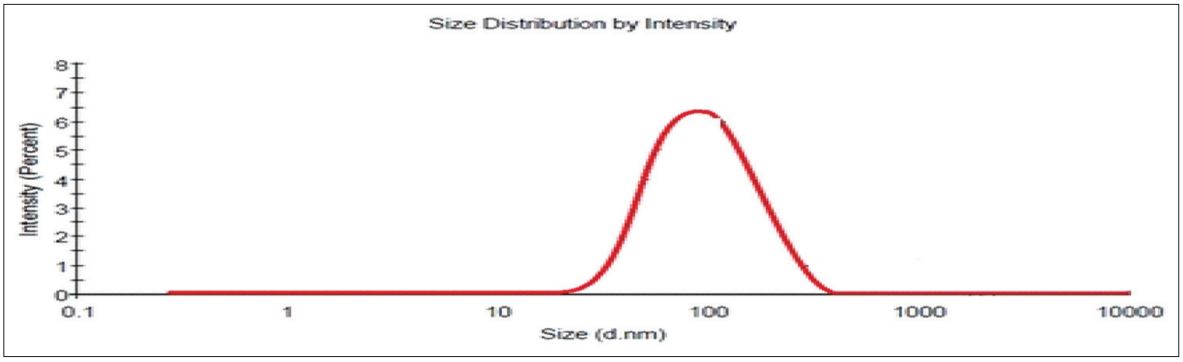

Figure 12: Particle size distribution of formulation EF-1

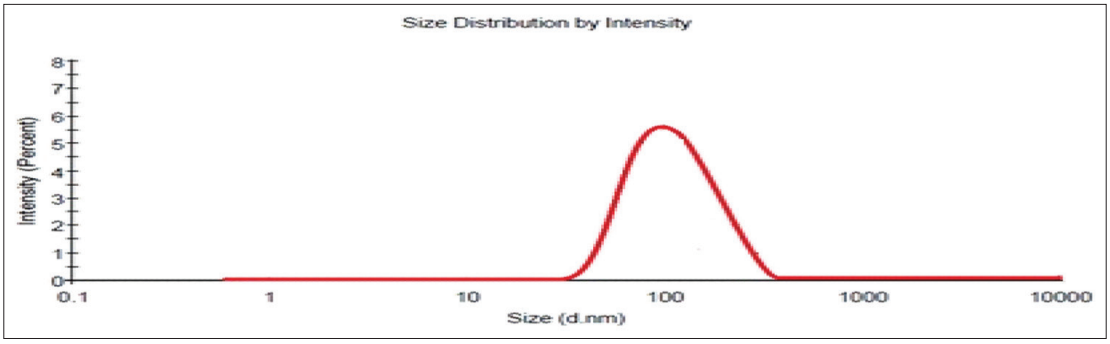

Figure 13: Particle size distribution of formulation EF-2

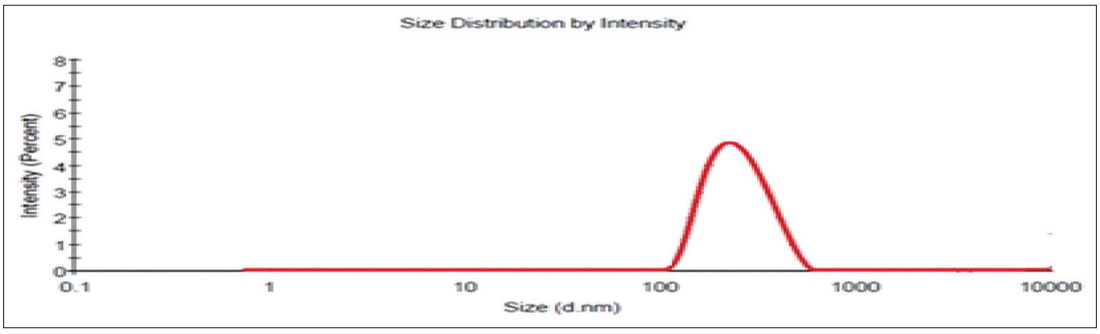

Figure 14: Particle size distribution of formulation EF-3

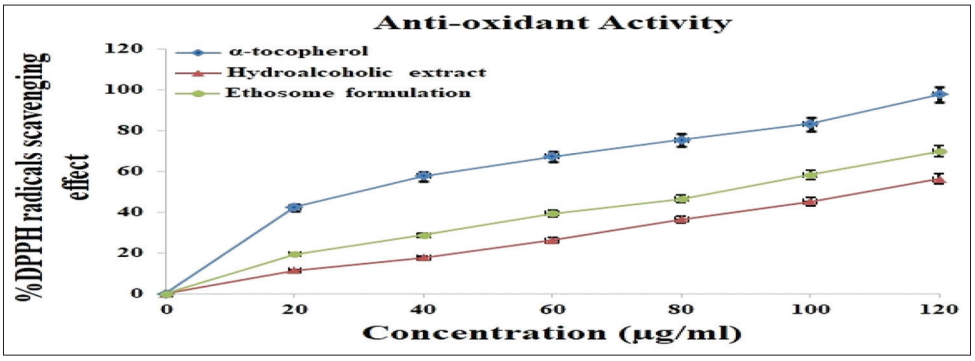

Figure 15: Dose-dependent scavenging of DPPH radicals by the hydroalcoholic extract of *Glycyrrhiza glabra* and compared with standard drug α-tocopherol. Each value represents mean±S.D. (*n*=3). The IC_{50} value of hydroalcoholic extract of *G. glabra*, ethosome formulation, and α-Tocopherol was 69.7±2.04, 56.2±1.89, and 97.6±2.04 µg/mL, respectively

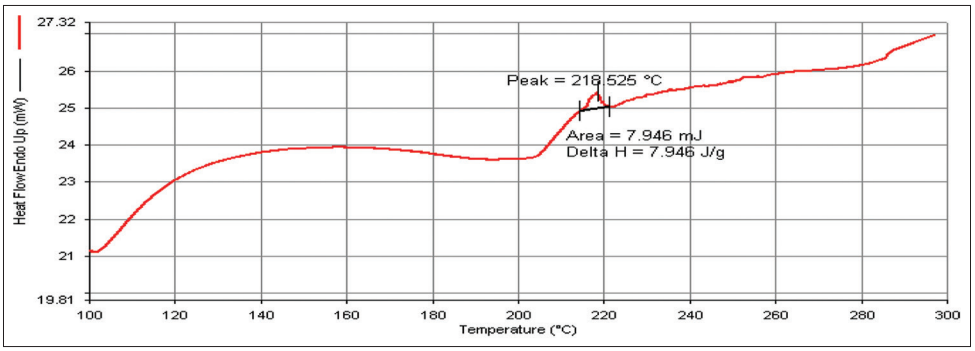

Figure 16: Differential scanning calorimetry spectra of glycyrrhizin

donates a hydrogen atom, it contributes to the reduced form of diphenyl picrylhydrazine (non-radical). The color of the reaction mixture changed from purple to yellow and its absorbance was measured at a wavelength of 280 nm. Standard α-tocopherol compound was taken to compare the antioxidant activity of hydroalcoholic extract of *G. glabra*. The IC_{50} of DPPH scavenging activity was calculated graphically [Figure 14]. Hydroalcoholic extract of *G. glabra* and ethosome formulation at a concentration of 150 µg/mL showed 69.7% and 56.2% activity comparable to that of positive control (α-tocopherol; 97.6%). It was found that the antioxidant activity of the hydroalcoholic extract was directly proportional to the concentration of extracts. A lower IC_{50} value indicates higher antioxidant activity. The antioxidant action may well be attributed to the presence of phenolic compounds and terpenes. The phytoconstituents existing in the extracts verified by the chemical test are rich in antioxidant principles that are responsible for anti-inflammatory, anti-acne, antifungal, and antimicrobial activities.[28] The antioxidant activity results are shown in Figure 15.

EE studies

Following centrifugation, the supernatant and pellet were separated, and the concentration of drug in both was analyzed by UV spectroscopic method.

Differential scanning calorimetry (DSC) analysis

DSC analysis was done on the standard drug to observe the melting point of the drug.

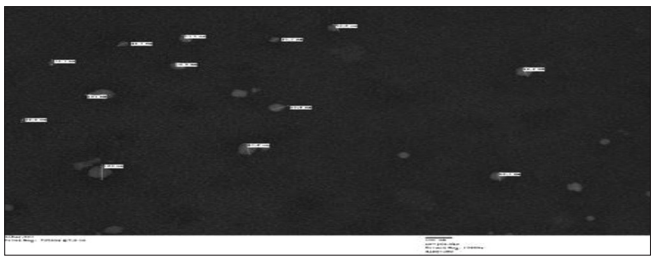

Figure 17: TEM microphotograph of optimized formulation

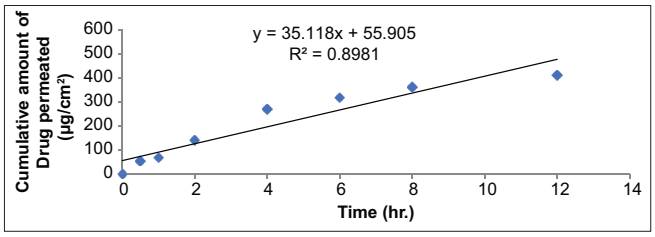

Figure 18: Drug release studies from hydroethanolic solution (control, C-1)

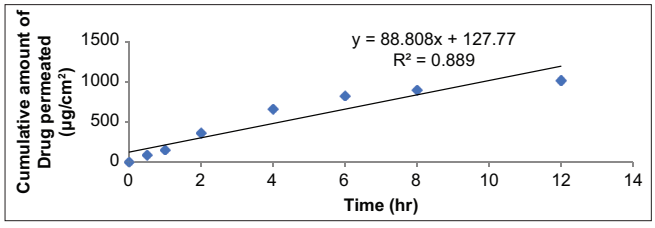

Figure 19: Drug release studies from optimized ethosomal formulation EF-2

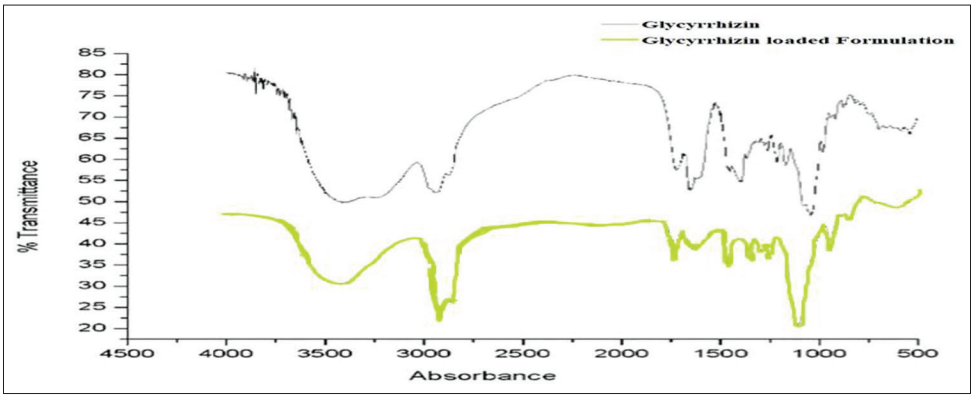

Figure 20: Fourier transform infrared spectrophotometry spectra of drug-loaded ethosomal formation

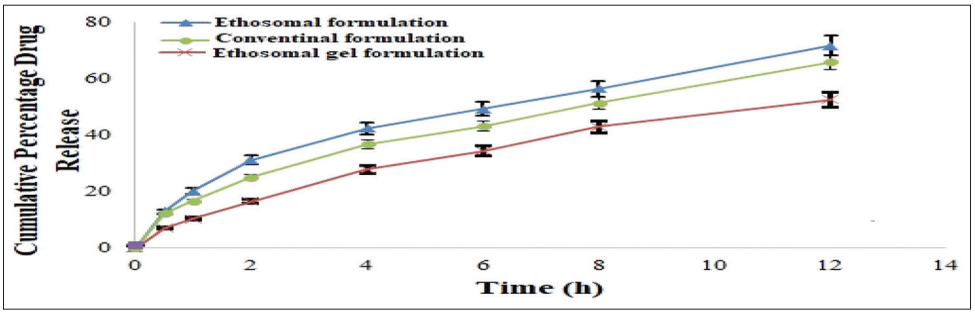

Figure 21: Comparative in *vitro drug* release profiles of optimized ethosome, ethosome gel, and conventional formulation

Vesicular shape and surface morphology

The TEM microphotograph of the optimized formulation finally concluded that the vesicles were spherical and finely distributed with the nano-range of vesicles [Figure 16].

Storage physical stability of ethosomes

Vesicle size measurements were performed on the drug-loaded ethosomes kept at three different temperatures. The stability of ethosomes was determined by monitoring the size of the vesicles. Results obtained indicated that the ethosomes were stable at room temperature (25°C) and 37°C for 90 days whereas the ethosomal formulation kept at 45°C had a slight increase in their vesicle size in the period of 60–90 days. It was concluded that the drug-loaded ethosomes were physically stable at varying temperatures and their vesicle size was in range, through at higher temperature (45°C), the vesicle size increased slightly over the 90 days Table 10.

In vitro permeation studies and drug retaining on skin surface

In vitro permeation studies were carried out using modified Franz diffusion cell to study the release of drug from the prepared vesicles and drug released from a hydroethanolic solution. The cumulative amount of drug permeated per unit area (μg/cm^2) was calculated and further Flux (μg/cm^2/h) was also calculated by plotting a graph between the cumulative amount of drug permeated (μg/cm^2) and time. Finally, after completion of the experiment, the drug content retained in the skin was evaluated using UV spectrophotometer.

FT-IR spectral analysis

The figure shows the FTIR overlay spectra of glycyrrhizin and its formulation. A detailed explanation of the FTIR spectroscopy of glycyrrhizin and its formulation is given in Table 13 and Figure 19. The loading of glycyrrhizin in ethosomal formulations has been reported without any chemical interactions.

Analysis of in vitro drug release kinetics

The in vitro release profile of the optimized ethosome, ethosome gel, and the conventional formulation was carried out. After 12 h, the %CDR from optimized ethosome formulations was found to be 71.64 ± 3.24% for optimized ethosome formulation, 52.608 ± 4.14% for ethosomal gel formulation, and 65.779 ± 2.65% for the conventional formulation, respectively [Figure 20]. The drug release kinetics evaluation for both the drugs showed best fitting Korsmeyer-Peppas model with higher R^2 values of 0.9872 for optimized formulation followed by Higuchi, first-order, and zero-order model, thus indicated the Fickian diffusion pattern of drug release. This *in vitro* drug release study exhibited that the optimized ethosomal formulation presents a suitable lipid matrix structure accomplished of entrapping drugs to assure their slow release, thus maintaining prolong the release of drugs and granting chemical stability to the formulation.

Conclusion

Acne vulgaris is a typical skin disorder and numerous formulations are available in the worldwide market, however, existing non-herbal formulations cause many side effects. In addition, the improvement of antibiotic resistance in acne-causing organisms has been rising steadily since the 1980s. The topical route of

application has great potential as an effective and safe way to administer glycyrrhizin-loaded formulation for its anti-acne effect in acne vulgaris. Glycyrrhizin was prepared in methanol and shows a positive significant correlation between the concentration and absorbance. Hence, the development of herbal topical formulation with effect is a very promising approach for its treatment. In the present study, *in vitro* permeation studies and drug retaining on skin surface confirm the efficacy of herbal ethosomal gel formulation comprising of methanolic extracts. It could be theorized that the developed topical herbal ethosomal gel formulation is suitable for the treatment of moderate-to-severe types of acne.

Authors' Contributions

Md. Imran Hyder made a substantial contribution to the conception, acquisition of data, and took part in the drafting of the article. Md. Arif Naseer and Adil Ahmad took part in revising it critically for important intellectual content, final approval of the version to be published.

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Conflicts of Interest

The authors declared that there are no conflicts of interest related to this study.

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