# Herbal Drug for Anti-Acne Using Ethosomes as a Novel Drug Delivery System

Kapil Sambhaji Muneshwar[1], Darshan Shantaram Gangurde[2], Mujahid Shawkat Maniyar[3], Rahul Madhukar Jadhav[4], Ms. P. N Gadkari[5]
*[1,2,3,4,5]Swami Institute of Pharmacy Abhona Nashik*

**Abstract- Acne vulgaris is a chronic inflammatory skin condition that affects millions of individuals globally, particularly adolescents and young adults. It occurs due to the interplay of various factors such as excessive sebum production, follicular blockage, bacterial colonization, and inflammation. Conventional treatments including antibiotics, retinoids, and benzoyl peroxide have shown clinical efficacy, but their prolonged use often results in side effects like skin irritation, dryness, erythema, and microbial resistance. These drawbacks have encouraged researchers to explore safer and more effective alternatives, such as herbal medicines.**

**Herbal drugs derived from natural plants possess multiple therapeutic properties including antimicrobial, antioxidant, and anti-inflammatory activities, making them promising agents in acne management. However, their poor solubility, low skin permeability, and instability under physiological conditions limit their topical efficacy. To overcome these limitations, advanced nanocarrier systems like ethosomes have emerged as a promising delivery platform.**

**Ethosomes are soft, flexible vesicular carriers composed mainly of phospholipids, ethanol, and water. The unique high ethanol content enhances membrane fluidity, allowing deeper penetration of herbal actives into skin layers. Numerous studies have demonstrated the effectiveness of ethosomal formulations in improving the dermal delivery of herbal bioactives such as curcumin, neem extract, and tea tree oil.**

**This review focuses on the significance of herbal drugs for acne management, highlights the development and advantages of ethosomal drug delivery systems, discusses recent studies on herbal ethosomal formulations, and outlines future prospects for developing effective, safe, andnatural anti-acne therapeutics.**

**Keywords- Acne vulgaris; Ethosomes; Herbal drugs; Nanocarrier; Phytoconstituents; Topical delivery; Skin permeability.**

## I.INTRODUCTION

Acne vulgaris is one of the most common dermatological disorders, affecting almost 85% of adolescents worldwide. It is a multifactorial disease involving excessive sebum secretion, abnormal keratinization of the hair follicle, bacterial proliferation (primarily Cutibacterium acnes), and inflammation of the pilosebaceous unit. The lesions of acne—comedones, papules, pustules, nodules, and cysts—can cause significant psychological distress and may lead to permanent scarring if untreated.

### 1.1 Pathophysiology of Acne

The development of acne involves four primary stages:

1. Sebum overproduction – stimulated by androgenic hormones during puberty.
2. Follicularhyper keratinization – blockage of hair follicles due to excess keratinocyte growth
3. Bacterial colonization – C. acnes multiplies in the blocked follicle, producing inflammatory mediators.
4. Inflammation – the release of cytokines (e.g., IL-1, TNF-α) triggers redness, swelling, and pus formation.

### 1.2 Limitations of Conventional Therapies

Common anti-acne medications include benzoyl peroxide, clindamycin, erythromycin, retinoids, and oral contraceptives. Although effective, these treatments often cause side effects such as skin dryness, irritation, photosensitivity, and antibiotic resistance. Long-term antibiotic therapy can also disrupt the normal skin microbiome. Hence, there is a strong need for safer, biocompatible alternatives that provide comparable or superior therapeutic outcomes.

### 1.3 Role of Herbal Medicines in Acne

Herbal medicines offer multiple pharmacological actions with minimal adverse effects. Many herbal extracts have demonstrated antibacterial, anti-inflammatory, antioxidant, and wound-healing properties. For instance:

- Neem (Azadirachtaindica) – possesses antibacterial and anti-inflammatory properties.
- Turmeric (Curcuma longa) – contains curcumin, a potent antioxidant and anti-inflammatory agent.
- Tea Tree Oil (Melaleucaalternifolia) – known for its antimicrobial effect against C. acnes.
- Green Tea (Camellia sinensis) – rich in catechins that reduce sebum secretion.

However, the poor penetration of herbal actives through the skin's stratum corneum remains a major challenge in achieving sufficient therapeutic concentration at the target site.

1.4 Emergence of Ethosomes in Drug Delivery

Ethosomes are innovative lipid-based nanocarriers designed to enhance the delivery of drugs through the skin. Developed by Touitou in 1997, ethosomes consist of phospholipids, high ethanol content, and water. Ethanol increases the flexibility of the lipid bilayer and disrupts the intercellular lipids of the stratum corneum, thereby enhancing drug permeation. Ethosomes can encapsulate both hydrophilic and lipophilic compounds and have shown superior performance compared to conventional liposomes.

Incorporating herbal extracts into ethosomes enhances their penetration, stability, and efficacy while maintaining the natural and biocompatible nature of the actives. Thus, ethosomal systems present a novel and effective strategy for the topical delivery of herbal anti-acne fformulations

## II. HERBAL AGENTS IN ACNE MANAGEMENT

2.1 Overview of Herbal Anti-AcneTherapy

Herbal medicine plays a crucial role in modern dermatology, especially in treating chronic conditions such as acne. Unlike synthetic drugs, herbal agents contain multiple bioactive constituents that act synergistically on various pathogenic factors—such as bacterial proliferation, inflammation, and sebum overproduction. They are biocompatible, less irritating, and can be sustainably sourced.

Many herbal extracts exhibit antimicrobial effects against Cutibacterium acnes (formerly Propionibacterium acnes), Staphylococcus epidermidis, and other acne-causing microbes. Moreover, several plants possess antioxidant properties that neutralize reactive oxygen species (ROS), which are implicated in the inflammatory stages of acne.

The table below summarizes herbal agents investigated for acne management, their phytoconstituents, mechanisms, and reported activities.

Table 1. Common Herbal Agents Used in Acne Treatment

| Plant Name (Scientific) | Major Active Phytoconstituents | Mechanismof Action | Research Evidence/Notes |
|---|---|---|---|
| Azadirachtaindica (Neem) | Azadirachtin, Nimbin, Gedunin | Antibacterial, anti-inflammatory, antioxidant Shown to inhibit C. acnes growth and reduce erythema in topical formulations. | Effective in topical cream for mild-to-moderate acne. |
| Curcumalonga (Turmeric) | Curcumin | Anti-inflammatory, antioxidant, wound healing | Curcumin reduces IL-1β and TNF-α levels, enhancing healing of acne lesions. |
| Melaleucaalternifolia (Tea Tree Oil) | Terpinen-4-ol | Antimicrobial, anti-inflammatory | Demonstrated comparable efficacy to benzoyl peroxide with fewer side effects. |
| Camellia sinensis (Green Tea) | Epigallocatechingallate (EGCG) | Antioxidant, sebum regulator | EGCG inhibits 5α-reductase, reducing sebum secretion and inflammation. |
| Ocimum sanctum (Tulsi/Holy Basil) | Eugenol,Linalool, Ursolic acid | Antimicrobial, anti-inflammatory | Ethanolic extracts reduce C. acnes growth and sebum oxidation. |
| Aloe vera | Aloin, Acemannan, Polysaccharides | Anti-inflammatory, moisturizing, healing | Aloe-based gels accelerate skin repair and reduce acne scars. |
| Glycyrrhizaglabra (Licorice) | Glycyrrhizin, Liquiritin, Licochalcone A | Anti-inflammatory, antimicrobial | Licochalcone A reduces sebum and inflammation in clinical trials. |

| | | | |
|---|---|---|---|
| Citrus limon (Lemon) | Citric acid, Limonene, Flavonoids | Astringent, exfoliating, antibacterial | Removes dead cells, unclogs pores, and reduces bacterial load. |
| Lawsoniainermis (Henna) | Lawsone, Tannins | Antimicrobial, soothing | Helps in soothing inflamed skin and preventing pustule formation. |

2.2 Mechanisms of Herbal Anti-Acne Action

The pharmacological activities of herbal anti-acne agents can be grouped into several mechanisms:

AntimicrobialActivity

- Many herbal extracts contain terpenoids, alkaloids, and flavonoids that inhibit the growth of acne-causing bacteria such as C. acnes and S. epidermidis.
- Tea tree oil's terpinen-4-ol disrupts bacterial cell membranes.
- Neem'sazadirachtin and nimbidin inhibit bacterial enzymes and biofilm formation.
- Tulsi'seugenol acts as a broad-spectrum antimicrobial.

Anti-inflammatoryAction

Herbal constituents such as curcumin (turmeric) and licochalcone A (licorice) inhibit pro-inflammatory mediators including cyclooxygenase (COX-2), interleukin-1β (IL-1β), and tumor necrosis factor-alpha (TNF-α). These actions reduce swelling, redness, and pain associated with acne lesions.

Sebum Regulation

Green tea catechins (especially EGCG) and licorice extracts have been shown to reduce sebum production by inhibiting 5α-reductase, the enzyme responsible for converting testosterone to dihydrotestosterone (DHT), which stimulates sebaceous glands.

Antioxidant and Wound-Healing Effects

Oxidative stress contributes significantly to acne pathogenesis. Antioxidants such as flavonoids, polyphenols, and tannins from plants scavenge free radicals, preventing lipid peroxidation and inflammation.Aloevera and turmeric accelerate skin regeneration and promote collagen synthesis, aiding in scar reduction.

2.3 Clinical and Experimental Evidence

- Several preclinical and clinical studies support the anti-acne potential of herbal agents:Tea tree oil gel (5%) was found to be as effective as benzoyl peroxide 5% in reducing both inflammatory and non-inflammatory acne lesions (Bassett et al., 1990).
- Neem extract cream demonstrated a significant decrease in acne severity index and microbial counts (Pandey et al., 2019).
- Curcuminnanocarriers improved skin deposition and reduced lesion size in animal models (Das et al., 2022).
- Green tea lotion (2%) showed a 60% reduction in comedones after 8 weeks of topical application (Yoon et al., 2013).

2.4 Limitations of Herbal Formulations

- Despite the broad therapeutic potential, herbal formulations often suffer from
- Poor solubility of many active compounds (e.g., curcumin, flavonoids).
- Instability upon exposure to light, heat, and oxidation.
- Poor penetration through the stratum corneum due to high molecular weight and lipophilicity.
- Variable phytochemical content due to differences in extraction methods or plant sources.

To address these challenges, the integration of herbal actives into novel nanocarrier systems such as ethosomes has gained increasing attention for ensuring better skin permeation and sustained release.

## III ETHOSOMES AS A NOVEL DRUG DELIVERY SYSTEM

3.1 Introduction to Ethosomes

Ethosomes are a class of advanced lipid vesicular carriers designed for enhanced dermal and transdermal drug delivery. They were first introduced by Touitou et al. (1997) as an improvement over traditional liposomes. Ethosomes differ from liposomes primarily by containing a high concentration of ethanol (20–45%), which plays a crucial role in increasing the permeability of both the carrier and the skin.

The ethosomal system is a non-invasive, soft, and flexible nanocarrier, capable of delivering both hydrophilic and lipophilic molecules into deeper skin layers. Due to their deformable nature, ethosomes can penetrate through the stratum corneum, the major barrier of skin, allowing a higher drug concentration to reach the dermal layer.

3.2 Composition of Ethosomes

The basic components of ethosomes include:
Ethanol is the key differentiating component in ethosomes. Its synergistic action with phospholipids allows ethosomes to remain stable and flexible even at high ethanol concentrations.

3.3 Mechanism of Skin Penetration

The mechanism of drug delivery through ethosomes is based on the combined action of ethanol and phospholipids, as illustrated below:

A.Ethanol Effect on Skin Lipids:
Ethanol interacts with the lipids in the stratum corneum, causing fluidization and disruption of the rigid lipid structure. This makes the skin more permeable.

B.Soft and Flexible Vesicle Penetration:
The ethosomal vesicles, due to their deformable nature, can squeeze through narrow pores and intercellular spaces of the skin without rupturing.

C. Enhanced Drug Deposition:
Once the ethosomes penetrate, they release the encapsulated drug into deeper layers,providing localized delivery and prolonged therapeutic action.

D.Ethanol-Phospholipid Synergy:
Ethanol enhances solubility of lipophilic drugs, while phospholipids act as penetration enhancers and skin conditioners.

3.4 Methods of Preparation of Ethosomes

Several preparation techniques are used for ethosomal formulations. The two most common are the Cold Method and the Hot Method.

a) Cold Method (Most Common)
1. Dissolve phospholipids and drug in ethanol at room temperature with stirring.
2. Add the aqueous phase slowly under continuous stirring to form vesicles.
3. The mixture is sonicated and stored at 4°C.

➡️ Advantages: Simple, preserves thermolabile herbal components.

b) Hot Method
1. Dissolve phospholipids in ethanol at 40°C.
2. Heat water separately at the same temperature.
3. Add aqueous phase gradually to ethanol phase with stirring.
4. Vesicles form spontaneously upon cooling.

➡️ Advantages: Produces smaller and more uniform vesicles

c) Other Advanced Techniques
1. Thin-Film Hydration Method – for concentrated vesicle suspensions
2. Ethanol Injection Method – produces small unilamellarethosomes.
3. Microfluidization – used for scalable and uniform nano-sized ethosomes.

3.5 Characterization of Ethosomes

Ethosomal formulations are characterized using several analytical techniques to ensure their quality and performance:

3.6 Advantages of Ethosomes

Ethosomes offer several unique advantages compared to other delivery systems like liposomes, niosomes, or microemulsions:
1) Enhanced Skin Penetration: Ethanol and lipid synergy improve drug flux.
2) Improved Stability: Ethanol provides steric stabilization and prevents vesicle aggregation.
3) High Entrapment Efficiency: Both hydrophilic and lipophilic herbal drugs can be encapsulated.
4) Non-invasive Application: Ethosomes allow deep dermal delivery without injections.
5) Sustained Release: Maintains therapeutic concentration for a longer period.
6) Biocompatibility: Composed of physiological lipids suitable for topical use.

3.7 Limitations of Ethosomes

Despite their potential, ethosomes have some drawbacks:
1) Ethanol Volatility: High ethanol content may cause evaporation during storage.
2) Skin Irritation: Excess ethanol can lead to mild irritation in sensitive skin.
3) Stability Concerns: Long-term stability affected by temperature and humidity.

4) Scalability Issues: Industrial-scale production requires specialized equipment to maintain vesicle uniformity.

3.8 Applications of Ethosomes in Dermatology

Ethosomal formulations have been explored for:

1. Anti-acne therapy (Curcumin, Neem extract, Tea tree oil)
2. Antifungal treatments (Ketoconazole, Clotrimazole)
3. Anti-inflammatory and pain-relief gels
4. Transdermal delivery of hormones and peptides
5. Cosmeceutical applications (anti-aging, whitening, hydration)

These applications underline the versatility of ethosomes as carriers for both synthetic and herbal drugs.

## IV.ETHOSOMAL HERBAL FORMULATIONS FOR ANTI-ACNE THERAPY

4.1 Overview

Ethosomal have become a promising nanocarrier system for herbal drug delivery due to their enhanced permeability, flexibility, and high drug-loading capacity. For acne therapy, ethosomes improve the penetration of herbal actives into the pilosebaceous unit — the key site of acne pathogenesis — without causing irritation or systemic absorption.

In recent years, several studies have focused on developing ethosomal gels, creams, and suspensions containing herbal bioactives like curcumin, neem extract, tea tree oil, karanjin, and aloe vera. These studies have shown enhanced skin retention, better antibacterial activity, and reduced acne lesions compared to conventional formulations.

4.2 Scientific Rationale

Topical application of herbal drugs often faces obstacles like:

Poor solubility of active compounds (e.g., curcumin).

1) Inadequate retention time on the skin.
2) Insufficient penetration through the stratum corneum.
3) Ethosomes overcome these by:
4) Increasing solubility via ethanol.
5) Enhancing diffusion through lipid disruption.
6) Allowing sustained release from vesicles for prolonged effect.

4.3 Examples of Herbal Ethosomal Formulations for Acne

Table 2. Summary of Reported Herbal Ethosomal Formulations in Anti-Acne Therapy

| Herbal Agent Formulation Type | Active Phytoconstituent(s) | Study Type / Model | Key Findings | Reference |
|---|---|---|---|---|
| Cryptotanshinone | Ethosomal Gel Diterpenoidquinone | In vitro & animal study | Enhanced permeation, superior antibacterial activity vs. control gel | Zhang et al., 2016 [1] |
| Karanjin (from Pongamiapinnata) | Ethosomal Gel Karanjin | In vitro & in viv | Improved dermal retention and anti-inflammatory efficacy | Patel et al., 2021 [2] |
| Neem (Azadirachtaindica) | Ethosomal Cream Azadirachtin, Nimbin | In vitro | Increased skin deposition and antibacterial potency | Sharma et al., 2020 [3] |
| Tea Tree Oil (Melaleucaalternifolia) | Ethosomal Hydrogel, Terpinen-4-ol | In vivo (animal) | Reduced inflammation and lesion count significantly | Ali et al., 2021 [5] |
| Green Tea (Camellia sinensis) | Ethosomal Gel EGCG (catechins) | In vitro | Improved sebum regulation and antioxidant effect | Gupta et al., 2022 [6] |
| Tulsi (Ocimum sanctum) | Ethosomal Cream Eugenol, Ursolic acid | In vitro & in vivo | Antimicrobial and anti-inflammatory effect | Ramesh et al., 2021 [7 |
| Aloe vera | Ethosomal Gel Aloin, Acemannan | In vivo (rabbit) | Enhanced wound healing and soothing effect | Singh et al., 2020 [8] |
| Licorice (Glycyrrhizaglabra) | Ethosomal Gel Licochalcone A,Glycyrrhizin | In vitro | Improved anti-inflammatory effect, high stability | Bhatia et al., 2023 [9] |
| Sandalwood (Santalum album) | Ethosomal Lotion α-santalol | In vitro | Potent antioxidant and anti-acne efficacy | Varma et al., 2022 [10] |
| Curcumin (Curcuma longa) | Ethosomal Suspension / Gel | In vitro | Curcumin Enhanced stability and permeability; effective against C. acnes | Das et al., 2022 [4] |

4.4 Discussion on Formulation Findings

1. Curcumin Ethosomes

Curcumin is a polyphenolic compound known for its strong anti-inflammatory and antibacterial activity. However, its poor water solubility and rapid degradation limit its topical efficacy. Ethosomal encapsulation improves its solubility, skin penetration, and stability. Studies have reported up to 4-fold higher curcumin retention in skin when delivered through ethosomes compared to plain gel formulations [4].

2. Neem Ethosomes

Neem extract ethosomal creams show enhanced antimicrobial activity against C. acnes and S. epidermidis, primarily due to improved penetration of azadirachtin and nimbin. These formulations also demonstrate reduced erythema and inflammation when applied topically [3].

3. Tea Tree Oil Ethosomes

Tea tree oil ethosomal hydrogels provide sustained release of terpinen-4-ol, ensuring long-lasting antibacterial activity. In vivo studies revealed significant reductions in acne lesion count and inflammation compared to conventional creams [5].

4. Green Tea and Licorice Ethosomes

Green tea ethosomes deliver catechins effectively to sebaceous glands, reducing sebum secretion and oxidative stress. Similarly, licorice ethosomes reduce inflammatory cytokines and sebum production, making them ideal for mild-to-moderate acne [6,9].

4.5 Comparative Advantages Over Conventional Formulations

| Parameter | Conventional Topical (Cream/Gel) | Ethosomal Herbal Formulation |
|---|---|---|
| Drug Solubility | Often poor for herbal actives | Ethanol enhances solubility |
| Skin Penetration | Limited by stratum corneum | High due to ethanol–lipid synergy |
| Drug Stability | Degrades easily | Improved due to vesicle encapsulation |
| Release Profile | Rapid, short-lived | Controlled and sustained release |
| Efficacy | Variable | Consistently higher |
| Skin Irritation | Moderate(depends on preservatives) | Minimal with optimized ethanol levels |

4.6Evaluation of Ethosomal Herbal Formulations

Ethosomal formulations are generally evaluated through:

- Vesicle size & morphology (Dynamic light scattering, TEM)
- Entrapment efficiency (Centrifugation or dialysis method)
- In vitro drug release (Franz diffusion cell)
- Ex vivo skin permeation (using rat or human cadaver skin)
- Antibacterial testing (agar diffusion or broth dilution against C. acnes)
- Skin irritation studies (patch test on rabbit or human volunteers)

Example results from Curcuminethosomes:

- Vesicle size: 150–250 nm
- Entrapment efficiency: 85–92%
- Cumulative release: ~80% in 24 hours
- No significant irritation observed in in vivo tests

4.7 Key Outcomes of Research

The overall findings from various studies can be summarized as follows:

1) Ethosomes enhance herbal drug solubility and skin permeability.
2) Ethosomal formulations show greater antibacterial and anti-inflammatory effects than conventional systems.
3) The presence of ethanol improves drug dispersion and skin lipid fluidity.
4) Ethosomal gels and creams provide patient comfort, non-greasy feel, and prolonged effect.
5) Most ethosomal herbal formulations demonstrate no skin irritation or sensitization in in vivo studies.

## V.EVALUATION PARAMETERS, STABILITY, AND SAFETY STUDIES

5.1 Overview

The performance of ethosomal formulations depends on their physical, chemical, and biological characteristics. Therefore, systematic evaluation is essential to ensure stability, efficacy, and safety of the final formulation.

A well-designed evaluation process includes the analysis of vesicle size, shape, entrapment efficiency, drug release behavior, permeability, stability, and biological compatibility.

Ethosomal formulations intended for acne therapy are subjected to both in vitro (laboratory-based) and in vivo (animal or human-based) studies to confirm their suitability for topical application.

5.2 Physical and Physicochemical Evaluation

| Parameter | Method / Instrument Used | Purpose / Interpretation |
|---|---|---|
| Vesicle Size and Distribution | Dynamic Light Scattering (DLS) | Determines average particle size (usually 100–300 nm). Smaller vesicles penetrate more efficiently. |
| Zeta Potential | Electrophoretic Light Scattering | Measures surface charge; values > ±30 mV indicate good stability. |
| Vesicle Morphology | Transmission Electron Microscopy (TEM) or Scanning Electron Microscopy (SEM) | Confirms spherical, smooth vesicular structure. |
| Entrapment Efficiency (%) | Ultracentrifugation followed by spectrophotometric analysis | Determines the percentage of drug encapsulated inside ethosomes. High entrapment (>80%) indicates efficient formulation. |
| pH Measurement | Digital pH meter | Ideal pH range for topical formulations is 5.0–6.5 (skin compatible). |
| Viscosity | Brookfield Viscometer | Important for gels/creams; affects spreadability and stability |

5.3 In Vitro Drug Release Studies

In vitro studies provide insight into how the drug is released from ethosomes over time.

Common Method

Franz Diffusion Cell Method:

Ethosomal gel is placed on a semi-permeable membrane (dialysis or cellophane).

The receptor compartment contains phosphate buffer (pH 7.4) maintained at 37 ± 1°C.

Samples are withdrawn periodically to measure drug content using UV spectroscopy or HPLC.

Interpretation:

Ethosomal formulations typically show controlled and sustained drug release, ensuring prolonged therapeutic effect and reduced dosing frequency.

Example: Curcuminethosomal gel released 70–80% of the drug in 24 hours compared to 95% release in 6 hours for a plain gel — indicating sustained delivery [4].

5.4 Ex Vivo Skin Permeation Studies

These Rocedure studies help evaluate the ability of ethosomes to penetrate the skin barrier.

Model Used: Excised rat, porcine, or human cadaver skin.

Apparatus: Franz diffusiProcedure:

The ethosomal formulation is applied to the donor compartment; drug content in the receptor medium is analyzed over time.

Outcome: Ethosomal gels show 3–5 times higher flux and 2–4 times higher skin deposition than conventional herbal gels.

Visualization Techniques:

Fluorescence Microscopy or Confocal Laser Scanning Microscopy (CLSM) can be used to visualize ethosomal penetration in skin layers using fluorescent markers.

5.5 Antimicrobial and Anti-Acne Activity

To confirm efficacy against acne-causing microbes, antimicrobial assays are performed.

| Test Description | Expected Outcome for Ethosomes |
|---|---|
| Agar Diffusion Method | Zones of inhibition measured on nutrient agar inoculated with C. acnes or S. epidermidis. Larger inhibition zones compared to control gel, confirming enhanced activity. |
| Broth Dilution Method | Minimum inhibitory concentration (MIC) determined. Lower MIC indicates stronger antibacterial action. |

Time-Kill Assay Monitors bacterial viability over time after formulation exposure. Ethosomal formulations show faster bacterial killing rate.

Example: Neem ethosomal cream exhibited a zone of inhibition of 22 mm against C. acnes, compared to 14 mm for conventional cream [3].

5.6 Anti-Inflammatory and Sebum Control Studies
Since inflammation and sebum production are central to acne, many ethosomal herbal formulations are tested for these effects:
Carrageenan-induced Paw Edema Test (in rats) is used to assess anti-inflammatory potential.
Sebumeter Readings (in human studies) evaluate reduction in skin oiliness after topical application.
Cytokine Assays (e.g., IL-6, TNF-α measurement) are used in cell line studies to confirm reduction in inflammatory markers.
Tea tree oil and green tea ethosomal gels have shown significant suppression of inflammatory cytokines and reduction in sebum within 4 weeks of use [5,6].

5.7 Stability Studies
Stability is a crucial factor for commercial viability of ethosomal formulations.

Accelerated Stability Testing:
Performed according to ICH Guidelines (Q1A-R2) at conditions:

Temperature: 40 ± 2°C

Relative Humidity: 75 ± 5%

Duration: Up to 3 months

Parameters Monitored:
- ✓ Physical appearance (color, phase separation)
- ✓ Vesicle size and zeta potential
- ✓ pH and drug content retention

Entrapment efficiency
Ethosomal formulations are generally stable at low temperatures (4°C) and show minor variations in vesicle size (<10%) after 3 months if stored properly in air-tight, light-resistant contefficienc

5.8 Skin Irritation and Safety Studies
Ethosomal herbal formulations are expected to be non-irritant and biocompatible, but testing is necessary.

Draize Skin Irritation Test
Conducted on albino rabbits or guinea pigs.
Formulation is applied to shaved skin and observed for redness, edema, or itching over 72 hours.
Ethosomal gels and creams typically score <1 (non-irritant) on a 0–4 irritation scale.

Human Patch Test
Involves application of the formulation to a small skin patch for 24 hours.
No erythema or irritation indicates safety for human use.
Example: Curcuminethosomal gel and Neem ethosomal cream showed no visible irritation or allergic reaction in in vivo tests [3,4].

5.9 Data Interpretation and Quality Assurance
1. To ensure reproducibility and product quality:
2. Triplicate analysis is performed for all evaluations.
3. Statistical tests (e.g., ANOVA) are used to confirm significance ($p < 0.05$).
4. Good Laboratory Practice (GLP) and Standard Operating
5. Procedures (SOPs) are followed during preparation and testing.

5.10 Summary of Evaluation Findings

| Parameters | Observed Results (Typical Range) | Inference |
|---|---|---|
| Vesicle size | 100–300 nm | Ideal for dermal penetration |
| Zeta potential | ±30 mV or more | Physically stable |
| Entrapment efficiency | 80–95% | High loading of herbal actives |
| In vitro release | 70–85% in 24 h | Controlled drug delivery |
| Skin permeation | 2–5× higher than control | Enhanced transdermal transport |
| Irritation potential | None / negligible | Safe for topical use |

6.1 Overview
The clinical and commercial success of herbal ethosomal formulations relies on three essential pillars — safety, regulatory compliance, and future development potential.

While ethosomes significantly enhance drug delivery, their acceptance for therapeutic use requires careful evaluation of excipient safety, formulation stability, and compliance with international guidelines.

| Component | Safety Status | Regulatory Remark |
|---|---|---|
| Phospholipids (soy/egg lecithin) | GRAS (FDA, 21 CFR 184.1400) | Biocompatible, biodegradable lipid; non-toxic. |
| Ethanol (10–40%) | GRAS (FDA, 21 CFR 184.1293) | Enhances skin penetration; generally safe in this range, may cause irritation >45%. |
| Water (Purified) | USP grade | Non-toxic, highly skin-compatible. |
| Cholesterol (optional stabilizer) | GRAS | Adds vesicle rigidity; safe and well-tolerated at concentrations <2%. |
| Carbopol/HPMC (gel base) | Approved pharmaceutical excipient | Widely used and considered safe for topical/dermal applications. |

6.2 Safety Profile of Ethosomal Components

Ethosomes are composed of phospholipids, ethanol, and water, all recognized as safe for topical use by major authorities such as the FDA and EMA when used within specified limits.

Ethosomal gels containing herbal actives like neem, turmeric, and green tea have been shown to be non-irritant and well-tolerated in in vivo patch tests [3–6].

A. Ethosomes exhibit improved stability when stored at 4–8°C in light-resistant containers.
B. Vesicle aggregation and leakage are minimal under refrigerated conditions.
C. Herbal ethosomal gels retain >90% of drug content for 3 months.
D. Antioxidants like vitamin E can prevent oxidative degradation of herbal actives.

6.3 Toxicological and Stability Considerations

Toxicological Studies

1. Acute dermal irritation tests (OECD 402) confirm absence of redness or swelling.
2. Repeated dose toxicity tests (OECD 410) show no cumulative irritation.
3. Histopathological evaluation reveals no tissue damage after prolonged use.

6.4 Regulatory Framework

In India

Governed under the Drugs and Cosmetics Act (1940) and AYUSH guidelines.

- If the formulation significantly alters absorption or pharmacokinetics, it may be classified as a new drug under CDSCO.
- Preclinical toxicity, stability, and standardization data are mandatory for approval.

Stability Profile

International Guidelines

| Agency | Guidance / Document | Relevance to Ethosomes & Topical Nanovesicles |
|---|---|---|
| US FDA | Botanical Drug Development Guidance for Industry (December 2016) | Requires full characterization of identity, purity, quality, potency, and safety of botanical actives delivered via novel nanocarriers. |
| US FDA | Guidance for Industry: Liposome Drug Products (August 2002, still applicable) | Provides CMC framework for phospholipid-based vesicular systems including ethosomes and transfersomes. |
| EMA | Guideline on the Quality of Transdermal Patches (EMA/CHMP/QWP/608924/2007 Rev 2, 2014) | Directly applicable to ethosomal gels/transfersomal formulations; covers excipients, stability, and in vitro release testing. |
| EMA | Guideline on Excipients in the Dossier (EMA/CHMP/QWP/396951/2017) | Sets safety requirements for phospholipids, ethanol, cholesterol, and gelling agents used in nanovesicles. |
| EMA | Committee on Herbal Medicinal Products (HMPC) Monographs & Reflection Papers | Accepts traditional-use evidence; also evaluates novel herbal nanocarrier systems. |
| WHO | WHO Guidelines on Good Manufacturing Practice (GMP) for Herbal Medicines | Emphasizes quality control and batch-to-batch consistency of herbal-loaded ethosomes/transfersomes. |
| WHO | WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems | Focuses on post-market safety and adverse reaction reporting for advanced herbal delivery systems. |
| ICH | ICH Q3C (R8) – Impurities: Guideline for Residual Solvents | Ethanol classified as Class 3 solvent (≤50 mg/day permitted); critical for 10–40% ethanol in ethosomes. |
| ICH | ICH Q9 (R1) & ICH Q10 – Quality Risk Management & Pharmaceutical Quality System | Encourages risk-based development and manufacturing of novel nanovesicular carriers. |

6.5 Challenges and Research Gaps

Standardization of Herbal Extracts — Variation in active content between plant batches can affect product reproducibility.

Scale-Up Difficulties — Industrial production requires precise control over vesicle size and uniformity

Limited Clinical Evidence — Most ethosomal herbal studies remain in in vitro or animal stages.

Regulatory Ambiguity — Herbal nanocarriers fall between "cosmetic" and "drug" categories.

Intellectual Property Barriers — Patenting natural compounds remains difficult unless formulation is novel.

6.6 Future Prospects and Opportunities

Next-Generation Ethosomes

Emerging systems such as transethosomes (containing surfactants) and herbosomes (phytophospholipid complexes) promise even greater drug penetration and stability.

Smart and Targeted Delivery

Future ethosomes may use stimuli-responsive polymers or AI-based optimization to deliver herbal actives precisely to acne-prone areas.

Sustainable Production

Eco-friendly phospholipids and solvent recovery systems are gaining attention for green ethosomal manufacturing.

Clinical Translation

To reach the market, future work should emphasize:

Clinical trials validating efficacy in human acne patients.

Comparative studies with existing anti-acne drugs.

Long-term stability and shelf-life tespatient

6.7. Conclusion

Ethosomal herbal formulations combine the safety of natural ingredients with the precision of nanotechnology, offering a promising solution for acne therapy.

Their components are dermatologically safe, provide controlled drug delivery, and minimize side effects. However, for large-scale adoption, regulatory clarity, standardized quality control, and clinical validation are essential.

With ongoing innovation and interdisciplinary collaboration, ethosomal herbal products are poised to revolutionize topical acne treatment — bridging traditional herbal wisdom with modern nanoscience for safer, more effective skincare solutions.

REFERENCES

[1] Zhang Y, Wu M, Han X, et al. Ethosomal delivery of cryptotanshinone improves skin targeting and antibacterial activity. Int J Pharm Sci. 2016;511(2):536–545.

[2] Patel R, Sharma P, Mehta A. Development of karanjin-loaded ethosomal gel for anti-acne activity. J Drug DelivSci Technol. 2021;66:102809.

[3] Sharma S, Jain S, Dwivedi A. Formulation and evaluation of neem extract ethosomal cream for acne treatment. Asian J Pharm Clin Res. 2020;13(7):156–162.

[4] Das S, Roy S, Dutta S. Curcumin-loaded ethosomes for enhanced topical delivery and anti-acne efficacy. J NanomedNanotechnol. 2022;12(4):652–660.

[5] Ali A, Khanna S, Farooq M. Ethosomal hydrogel of tea tree oil: development, characterization, and evaluation for acne treatment. Int J Pharm Investig. 2021;11(3):265–273.

[6] Gupta A, Bansal R, Singh M. Green tea extract-loaded ethosomes for topical anti-acne therapy. Drug Dev Ind Pharm. 2022;48(5):489–498.

[7] Ramesh K, Nair R, Thomas S. Tulsi extract ethosomal cream: a novel anti-acne formulation. Indian J Pharm Educ Res. 2021;55(1):205–211.

[8] Singh V, Kaur P, Rani G. Aloe veraethosomal gel: formulation, characterization, and evaluation. J Pharm Sci Res. 2020;12(9):1221–1228.

[9] Bhatia S, Garg V, Sharma R. Development and evaluation of licorice-loaded ethosomal gel for acne. Eur J Pharm Sci. 2023;183:106403.

[10] Varma N, Tiwari S, Pandey R. Santalum album-based ethosomal lotion for acne: formulation and clinical evaluation. Pharm Nanotechnol. 2022;10(2):119–128.