International Journal of Scientific Research & Engineering Trends
Volume 12, Issue 2, Mar-Apr-2026, ISSN (Online): 2395-566X

Formulation and Evaluation of Ethosomes from Drimia Indica Species

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Abstract- Traditional herbal medicines serve as a primary healthcare pillar for approximately 80% of the population in various Asian and African nations. Despite their extensive experiential evidence and therapeutic benefits, conventional herbal formulations face significant pharmacokinetic limitations. These include poor aqueous solubility, unstable gastrointestinal pH degradation, high presystemic metabolism, and an inability to cross lipid biomembranes effectively, often resulting in sub-therapeutic blood levels.[1] Modern quality control has transitioned from single-marker assays to comprehensive metabolic profiling using High-Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-MS) and genomic DNA barcoding for precise species identification. Concurrently, international bodies (including the WHO, ASEAN, EU, and FDA) are collaborating to harmonize regulatory frameworks. To enhance therapeutic efficacy, nanotechnology is being deployed to engineer nano-phytomedicines. Various carrier systems including polymeric nanoparticles, solid lipid nanoparticles, liposomes, nanoemulsions, and phytosomes are evaluated. Notably, while liposomes encapsulate extracts within an aqueous core or lipid bilayer, phytosomes chemically anchor phytochemicals directly to phospholipid head groups, drastically improving lipophilicity and membrane permeation.[2] Incorporating plant actives into nanostructured matrices significantly optimizes their hydrophilic-lipophilic balance. This structural modification provides sustained release, shields molecules from chemical degradation, minimizes off-target toxicity (e.g., localized accumulation of chemotherapeutics in healthy tissues), and increases bioavailability. However, transitioning these formulations from bench to industrial scale introduces complex challenges, including maintaining uniform encapsulation efficiency within multi-component plant extracts, preventing nanoparticle aggregation driven by high surface energy, and satisfying stringent regulatory safety assays regarding tissue accumulation.[3].

Keywords – Phytoformulations; Nano-phytomedicines; Phytosomes; Bioavailability; Novel Drug Delivery Systems (NDDS); Standardization.

I. INTRODUCTION

An herbal drug is to be defined as a drug intended for disease treatment, preventions, safety for human use in the day to day life. In an Asian country herbal drug play a vital role in human health care tradition. A Different dosage form of herbal drug formulation is used into health modern science of technology in human healthcare purposes. According to The World Health Organization estimates that 80 percent of populations of some Asian and African countries presently use herbal medicine for purpose of treatment and primary health care purpose worldwide. The herb word is derived from Latin word herb herbs and old French world here. Medicinal plants such as aloe, tulsi, turmeric, and ginger. They are mainly considered as home remedies in many parts of the country. Herbal medicine has great advantages in evolved throughout the time of human civilization. [1],[5]

Herbal drugs constitute a major share of all the officially recognised systems of health in India viz. Currently, there is no separate category of herbal drugs or dietary supplements, as per the Indian Drugs Act. However, there is a vast experiential evidence base for many of the natural drugs. This offers immense opportunities for Observational Therapeutics and Reverse Pharmacology. [2]

In this regard, the WHO has formulated standard procedures and methods for standardizing herbal medicines. Despite conventional techniques outlined in the WHO document, novel techniques including barcoding, protein chip, metabolomics, genomic fingerprinting, analytical compound examination,

spectroscopy and so, on have been arriving in the last decades for the herbs/herbal product standardization 9. Elsewhere, WHO and other concerned regional bodies, namely, ASEAN, European Union (EU), United States of America (USA), and United Kingdom (UK), are taking collaborative maneuvers to establish a single regulatory framework for the safety, efficacy, and standardization of herbal medicinal product.[3]

By administering herbal medicine in the nano-size form, there are chances for improving the bioavailability, binding receptor selectivity due to higher active surface energy thereby enhancing the effectiveness and safety of the active entity. In the last few decades, formulations with nano-sized herbal active ingredients have emerged as nano-phytomedicines owing to its wide range of interest and effectiveness because of its unique nature. Nanonized drug delivery structure of herbal drug has an approaching outlook for getting bigger the doings and overcome problems associated with plant medicine. The current review will focus on nanoparticles, herbal drug-loading techniques, herbal nanoformulations, and applications in various fields.[4]

To complement these shifting frameworks, researchers are implementing high-throughput screening and multi-marker fingerprinting. Unlike classic single-marker assays which often fail to reflect the total therapeutic profile of an extract, modern quality control relies heavily on High-Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-MS) to generate metabolic profiles. Furthermore, DNA barcoding assists in identifying exact botanical species, actively preventing intentional or accidental adulteration with look-alike plant species before the extraction process ever begins.

In phytoformulation research, developing nano-sized dosage forms (polymeric nanoparticles and nanocapsules, liposomes, solid lipid nanoparticles, phytosomes, and nanoemulsion) has a number of advantages for herbal drugs, including enhancement of solubility and bioavailability, protection from toxicity, enhancement of pharmacological activity, enhancement of stability, improving tissue macrophage distribution, sustained delivery, and protection from physical and chemical degradation. Thus, the nanosized NDDS of herbal drugs have a potential future for enhancing the activity and overcoming problems associated with the plant medicines. Liposomes, which are biodegradable and essentially nontoxic vehicles, can encapsulate both hydrophilic and hydrophobic materials.[5]

To understand how these specific carriers improve bioavailability, it is useful to look at their structural differences. Natural phytochemicals struggle to pass through cellular lipid membranes due to their physical structures. Carriers like liposomes and phytosomes solve this differently, a liposome encapsulates the active extract inside an inner aqueous core or within its lipid bilayers. In contrast, a phytosome forms a direct chemical complex where the active plant components anchor straight to the polar head groups of phospholipids. This direct bonding creates a highly lipid-soluble complex that diffuses through biological membranes with much higher efficiency.

In the past few decades, considerable attention has been concentrated on the evolution of an NDDS for herbal drugs. Herbal drugs are getting more popular in the modern world for their diligence to cure a variety of diseases with less toxic effects and better therapeutic effects. Meanwhile, some limitations of herbal extracts/plant actives such as instability in highly acidic pH and liver metabolism have gone to attain the drug levels below to the therapeutic concentration in the blood resulting in less or no healing effect. Incorporation of novel drug delivery technology to herbal or plant actives minimizes the drug degradation or presystemic metabolism and serious side effects by accumulation of drugs to the non-targeted areas and improves the ease of administration in the pediatric and geriatric patients. [6]

Conventional dosage forms, including prolonged-release dosage forms, are unable to fulfill the ideal requirements of novel carriers such as ability to deliver the drug at a rate directed by the penury of the body and to transmit the active entity of herbal drug to the site of activity. For good bioavailability, natural products must have a sound balance between hydrophilicity (for dissolving into the gastrointestinal fluids) and lipophilicity (to cross lipidic biomembranes). Novel herbal drug carriers cure particular disease by targeting just the affected zone inside a patient's body and transporting the drug to that region.

Despite these clear benefits, moving nano-phytomedicines from small laboratory experiments into mass industrial production involves a few major challenges. Because raw plant extracts are naturally complex mixtures containing dozens of different compounds, achieving uniform encapsulation efficiency so that every single nanoparticle contains the exact same ratio of active ingredients is incredibly difficult.[9]

Additionally, when materials are brought down to the nanometer scale, their high surface energy can lead to physical instability, causing particles to clump together or aggregate over time. Finally, the regulatory approval of these nano-carriers requires strict new safety assays to confirm that the nanomaterials break down safely and do not build up permanently within non-targeted liver or spleen tissues.

NDDS is advantageous in giving up the herbal drug at predetermined rate and delivery of drug at the site of action which minimizes the toxic effects with an increase in bioavailability of drugs. In novel drug delivery technology, control of the dispersion of the drug is achieved by incorporating the drug in carrier system or in modifying the social organization of the drug at the molecular level.

Incorporation of herbal drugs in the delivery system also aids to increase in solubility, enhanced stability, protection from toxicity, enhanced pharmacological activity, improved tissue macrophage distribution, sustained delivery, and protection from physical and chemical degradation. For example, liposomes act as potential vehicles to take anticancer agents by increasing amount of drug in tumor area and decrease the exposure or accumulation of drug in normal cells/tissues, thereby preventing tissue toxicity effects.[10]

The phytosomal carriers have been considered for effective delivery of herbal extracts of ginseng (Ginkgo biloba), etc. Direct binding of phosphatidylcholine to herbal extract components led to better absorption characteristics as compared to conventional delivery of herbal infusions. Other vesicular assemblies such as microspheres, animations, and polymeric nanoparticles have been shown beneficial to carry herbal components.

An herbal drug is systematically defined as any plant-derived formulation intended for the mitigation, treatment, or prevention of diseases, while maintaining a verified safety profile for human consumption in daily life. Across Asian and African nations, botanical therapeutics form the foundational bedrock of traditional healthcare ecosystems. Rather than remaining confined to historical folklore, diverse dosage forms of herbal formulations are being aggressively integrated into modern biomedical science and technological frameworks.[12]

The global footprint of these therapies is immense; the World Health Organization (WHO) estimates that approximately 80% of the population in select Asian and African countries relies on herbal medicine for their primary healthcare needs. Etymologically, the word "herb" traces its lineage back to the Latin herba and the Old French erbe. Universally recognized medicinal flora—including Aloe vera (aloe), Ocimum sanctum (tulsi), Curcuma longa (turmeric), and Zingiber officinale (ginger)—have transitioned from fundamental home remedies into highly scrutinized raw materials for modern pharmacy, proving that botanical medicine has evolved in tandem with the trajectory of human civilization. [10]

Within the Indian pharmaceutical matrix, herbal drugs constitute a major share of all officially recognized traditional systems of medicine (under the AYUSH framework). Historically, the Indian Drugs and Cosmetics Act has lacked a sharply isolated, separate category for complex herbal extracts versus dietary supplements. However, the vast repository of experiential, empirical evidence supporting natural products offers a profound launchpad for Observational Therapeutics and Reverse Pharmacology—a paradigm where clinical efficacy is validated first, followed by downstream mechanistic and laboratory exploration.

To harmonize this sector globally, the WHO has established strict, standardized procedures for evaluating herbal medicines. Moving beyond conventional macroscopic and botanical techniques, the last few decades have witnessed an analytical revolution. Modern standardization relies heavily on cutting-edge methodologies, including:

- Genomic Fingerprinting & DNA Barcoding: Actively identifying exact botanical species to prevent accidental or fraudulent adulteration with look-alike species before extraction begins.
- Metabolomics & High-Throughput Screening: Utilizing High-Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-MS) to map multi-marker metabolic fingerprints, capturing the total therapeutic profile of an extract rather than misleading single-marker assays.
- Spectroscopic Profiling & Protein Chips: Mapping complex macromolecular interactions within the herbal matrix.

Concurrently, international regulatory bodies—including the WHO, ASEAN, the European Union (EU), the United States Food and Drug Administration (USFDA), and the United Kingdom's MHRA—are actively collaborating to bridge geopolitical gaps. Their collective goal is to establish a singular, unified regulatory framework that mandates strict safety, efficacy, and standardization protocols for all herbal medicinal products in global commerce. [14]

A critical limitation of raw plant extracts or isolated phytochemicals is their poor biopharmaceutical profile. Many potent natural products fail to exhibit in vivo efficacy due to instability in the highly acidic gastric pH, rapid hepatic first-pass metabolism, low aqueous solubility, or an inability to cross lipophilic bio membranes. To achieve therapeutic efficacy, an active compound must maintain a delicate balance between hydrophilicity (to dissolve in gastrointestinal fluids) and lipophilicity (to partition across cellular lipid bilayers). [14]

By engineering herbal actives into nano-sized delivery architectures 100 researchers have birthed the field of nano-phytomedicines. Administering botanical therapeutics at the nanoscale dramatically alters their pharmacokinetics by:

1. Elevating Surface Free Energy: Increasing the active surface area, which drastically improves dissolution rates and binding receptor selectivity.
2. Overcoming Physiological Barriers: Maximizing systemic bioavailability and shielding sensitive phytochemicals from presystemic metabolism.
3. Targeting Site-Specific Zones: Directing drugs preferentially to affected biological tissues while avoiding healthy, non-targeted organs, thus reducing systemic toxicity.
4. While the benefits of nano-phytomedicines are clear in laboratory settings, translating these formulations from

small-scale academic benches to large-scale industrial manufacturing presents major hurdles. [15]

- Encapsulation Homogeneity: Because crude plant extracts are inherently heterogeneous mixtures containing dozens of co-existing phytochemicals, achieving uniform encapsulation efficiency—where every single nanoparticle maintains the exact same stoichiometric ratio of active ingredients—is exceptionally difficult.
- Physical Aggregation: Due to their incredibly small scale, nanoparticles possess high surface free energy. This creates a strong thermodynamic drive to reduce surface area, frequently causing the particles to clump, flocculate, or aggregate over time in storage, ruining formulation stability.
- Toxicological Clearance: The regulatory approval of nano-carriers demands exhaustive, novel bioassays. Researchers must definitively prove that these synthetic or natural nanomaterials undergo safe biodegradation and excretion, rather than causing long-term, toxic bioaccumulation within reticuloendothelial tissues like the liver and spleen. [16]

TYPES OF NOVEL HERBAL DRUG DELIVERY SYSTEMS:

Several approaches in case of new herbal drug delivery system include different types of expressions such as

- Mouth-dissolving tablets
- Liposomes
- Phytosomes
- Controlled-release formulation
- Nanoparticles
- Microspheres
- Transfersomes
- Proniosomes
- Transdermal drug delivery system (TDDS)
- Ethosomes

Mouth-dissolving tablets

Asoka Lifescience Limited launched Res-Q, the world's first polyherbal mouth-dissolving tablet, fast mouth-dissolving drug. It induces a new drug delivery system that imparts increased efficacy. In the Ayurvedic medicine segment, this is the inaugural attempt to make medicines more effective in managing chronic ailments. Res-Q is a polyherbal medicine highly effective for lung problems and other respiratory ailments such as asthma. This unique mouth-dissolving drug delivery system ensures that the drug reaches the blood right away and the first-pass metabolism is bypassed. It dissolves in mouth by mixing with the saliva and get absorbed. This Res-Q provides relief from respiratory distress within 15 min. Hence the product shows a great resemblance with the efficacy of Sorbitrate, a revolutionary mouth-dissolving drug used in cardiac distress. [19]

- Mechanism of Fast Disintegration: Mouth-dissolving tablets (MDTs) rely heavily on superdisintegrants (like cross-linked polyvinylpyrrolidone or sodium starch glycolate) or highly water-soluble excipients. When exposed to a minimal volume of saliva, these agents cause the tablet to rapidly swell or wick water, breaking it apart within seconds without requiring water or chewing.
- The Pre-Gastric Absorption Advantage: Because the mucosal lining of the oral cavity is highly vascularized, lipophilic active phytochemicals can directly enter the systemic capillary bed via the sublingual and buccal routes. This completely bypasses the harsh gastric acids of the stomach and the destructive first-pass hepatic metabolism in the liver, drastically speeding up the therapeutic response time.
- Clinical Relevance of Res-Q and Sorbitrate: In acute conditions like an asthma attack or cardiac angina (traditionally managed by synthetic vasodilators like Sorbitrate), rapid blood-plasma saturation is the difference between tissue recovery and severe distress. Achieving a 15-minute response time with a polyherbal blend represents a major leap in alternative emergency therapeutics. [17]

Liposomes

Liposomes are self-assembled (phospho)lipid-based drug vesicles that form a bilayer (uni-lamellar) and/or a concentric series of multiple bilayers (multilamellar) enclosing a central aqueous compartment. The size of liposomes ranges from 30 nm to the micrometer scale, with the phospholipidbilayer being 4–5 nm thick. The field of liposomology was launched by the British scientist Alec Bangham and colleagues at Babraham Cambridge in the mid-1960s, and they first published the structure of liposomesin 1964. Since then, liposomes have been widely investigated as delivery vehicles for small molecular drugs, protein, nucleic acid, and imaging agents. [18]

- Structural Versatility: Liposomes are highly versatile because they possess a dual nature: a central hydrophilic (water-loving) core and surrounding lipophilic (fat-loving) phospholipid bilayer membrane. This allows them to carry water-soluble antioxidants (like Vitamin C or polyphenols) in their core, while simultaneously embedding lipid-soluble plant actives (like essential oils or carotenoids) directly into the oily membrane walls.
- Surface Modification (Stealth Liposomes): Standard liposomes are easily recognized and cleared out of the blood by the body's immune defense system (the reticuloendothelial system). To prevent this, modern liposomes are coated with hydrophilic polymers like Polyethylene Glycol (PEGylation). This creates a "stealth" shield that blocks immune detection, extending the drug's circulation time from minutes to several hours.
- Passive and Active Targeting Mechanisms: Liposomes can naturally gather in inflamed or cancerous tissues through

microscopic gaps in leaky blood vessels a process called the Enhanced Permeability and Retention (EPR) effect. Furthermore, they can be engineered with specific homing molecules (like antibodies or ligands) attached to their surface to selectively bind to targeted diseased cell receptors, sparing healthy tissues. [19]

Phytosomes

Phytosomes means herbal drug loaded in vesicles, which is available in the Nano form. The phytosome provide an envelope, like coating around the active constituent of drug and due to this the chief constituent of herbal extract remains safe from degradation by digestive secretion and bacteria. Phytosome is effectively able to absorb from a water loving environment into lipid loving environment of the cell membrane and finally reaching to blood circulation. The current review highlights the future scope and emerging technologies in the field of NDDS for the benefit of herbal and traditional medicines prepared from plant origins. The term “Phyto” means plant and “some” means cell like. It is also mentioned as herbosomes. Phytosomes can easily traverse the lipophilic path of the enterohepatic cell membranes and also stratum corneum layer of the skin. [20]

- The Nature of the Molecular Complex: Unlike liposomes, where the plant extract is loosely floating inside a central pocket or lipid layer, a phytosome is a distinct, stoichiometric molecular complex. The active phytochemicals (typically polyphenols, flavonoids, or terpenoids) form actual hydrogen bonds with the polar head groups of dietary phospholipids (like phosphatidylcholine), turning the entire plant extract into an easily absorbed lipid unit.
- Dramatically Superior Bioavailability: Many potent herbal bioactives are highly water-soluble but cannot cross the fatty, lipid-rich cellular barriers of the gut wall. By wrapping these compounds in a lipid-friendly wrapper, phytosomes allow them to effortlessly pass through the lipophilic cell membranes of the gastrointestinal tract, leading to blood absorption levels that can be up to 3 to 5 times higher than standard raw plant extracts.
- Topical and Transdermal Benefits: Because phytosomes easily slide through lipid pathways, they are uniquely suited for topical skin applications. They can effectively traverse the lipid-heavy stratum corneum (the outermost protective layer of skin), allowing deeper delivery of anti-inflammatory or anti-aging herbal compounds into the dermis without requiring chemical skin-peeling agents. [21]

Controlled-release formulation

A patent describes an orally administrable formulation for the controlled release or stable storage of a granulated herb, comprising a granulated herb and a carrier, the formulation release of 75% of the active ingredients between 4 and 18 h after administration. The active elements are selected from the group consisting of hypericin, hyperforin, and echinacosides. The invention seeks to provide improved herbal preparations, whose preparations offer a convenient oral dosage form of herbs for supplying optimum plasma concentrations of the biologically active compounds that facilitates user compliance.

Microgranules can be cleared up by a number of different operations, for example, extrusion–spheronization, fluid–air bed process, or a cutting-pan method. Extrusion–spheronization is suitable for pellets with high content of active meaning, but need more equipment. For the manufacture of the granules of the invention, the cutting-pan method is preferred, as it requires only simple equipment and procedure. [22]

- Maintaining the Therapeutic Window: The main goal of a controlled-release herbal formulation is to keep the active phytochemicals inside a tight therapeutic window—high enough in the blood to heal the body, but low enough to avoid toxic side effects. This eliminates the "peak-and-valley" effect caused by conventional teas or immediate-release capsules, which spike and fade rapidly.
- The Chemistry of Matrix Excipients: To achieve a slow, steady release over a 4 to 18-hour window, microgranules are bound within specialized swelling hydrophilic polymers (like Hydroxypropyl Methylcellulose, or HPMC) or insoluble hydrophobic wax matrices. As gastrointestinal fluids slowly penetrate the granule, the polymer forms a thick gel barrier, forcing the active herbs to diffuse out into the body at a highly controlled, predictable rate.
- Advantages of the Cutting-Pan Manufacturing Process: While high-end extrusion-spheronization creates perfectly round pellets, it requires expensive, specialized machinery and high energy inputs that can degrade sensitive herbs. The cutting-pan method uses simple rotational tumbling and spraying coats to layer herbal extracts onto core seeds. This low-heat, cost-effective method is ideal for scaling up traditional herbal medicine manufacturing in developing regions without altering the fragile chemical components of the plants. [23]

Nanoparticles

Nanoparticles are nanometer-sized particles that are nanoscale in three dimensions. They include nanopores, nanotubes, quantum dots, nanoshells, dendrimers, liposomes, nanorods, fullerenes, nanospheres, nanowires, nanobelts, nanoringsand nanocapsules.

The applications of nanoparticles include drug-delivery systems, cancer targeting, dentistry, etc. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structures. A bulk material should have constant physical properties regardless of its size, but at the nanoscale this is often not the case. Size-dependent properties are observed, such as quantum confinement in semiconductor particles, surface plasmon

resonance in some metal particles, and super paramagnetism in magnetic materials.
Nanoparticles exhibit a number of special properties relative to bulk material. Nanoparticles often have unexpected visual properties because they are small enough to confine their electrons and produce quantum effects. For example, gold nanoparticles appear deep red to black in solution. The often very high surface area to volume ratio of nanoparticles provides a tremendous driving force for diffusion, especially at elevated temperatures. Sintering is possible at lower temperatures and over shorter durations than for larger particles. [24]

Nanoparticles are solid, particulate structures ranging in size from 100 in at least one dimension, though strictly defined in nanotechnology as being nanoscale in all three spatial dimensions. They serve as a fundamental structural class that encompasses a vast array of morphologies, including:

- Carbon-based structures: Nanotubes (CNTs) and fullerenes (60).
- Core-shell configurations: Nanoshells and nanocapsules.
- Symmetric geometries: Nanospheres, quantum dots, and nanorods.
- Complex architectures: Dendrimers (highly branched polymers), liposomes (lipid bilayers), nanowires, nanobelts, and nanorings.

The Physics of the Nanoscale: Bridging Bulk and Atoms

Nanoparticles are of profound scientific interest because they occupy a transitionary zone acting as a physical bridge between bulk materials and discrete atomic or molecular structures. While bulk materials maintain uniform physical, optical, and chemical properties regardless of size, materials at the nanoscale undergo drastic changes due to two primary phenomena:

- Quantum Confinement Effects: As particle size decreases to the nanoscale, the electronic wave functions of the material are spatially confined. This alters the electronic band structure, causing semiconductor particles (quantum dots) to emit different colors of light purely based on their size.
- Surface Plasmon Resonance (SPR): In noble metal nanoparticles (like gold or silver), the conduction electrons resonate collectively when stimulated by specific wavelengths of incident light. Consequently, gold nanoparticles do not appear metallic yellow; instead, they exhibit deep red, purple, or black hues in solution depending on their size and shape.

Thermodynamic Properties
Because of their miniscule size, nanoparticles possess an exceptionally high surface-area-to-volume ratio. A massive percentage of the material's atoms reside on its surface rather than its core, leading to high surface free energy. This creates a powerful thermodynamic driving force for diffusion. As a result, phenomena like sintering (the coalescing of solid particles without melting) occur at substantially lower temperatures and significantly shorter durations compared to bulk macro-particles.

Biomedical Applications
In modern medicine, nanoparticles are engineered for:

- Targeted Drug Delivery Systems (DDS): Encapsulating therapeutic agents to protect them from premature degradation.
- Oncology (Cancer Targeting): Utilizing the Enhanced Permeability and Retention (EPR) effect to passively accumulate in tumor tissues, or using ligands for active cellular targeting.
- Advanced Dentistry: Integrating antimicrobial nanoparticles (e.g., silver or zinc oxide) into dental composites and bone regeneration scaffolds. [23]

Microspheres
Microspheres are solid spherical particles ranging in size from 1-1000µm. They are spherical free flowing particles consisting of proteins or synthetic polymers. The microspheres are free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature.

There are two types of microspheres;

- Microcapsules.
- Micromatrices.

Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall and micromatrices in which entrapped substance is dispersing throughout the microsphere matrix. Solid biodegradable microspheres incorporating a drug dispersed or dissolved through particle matrix have the potential for the controlled release of drug. They are made up of polymeric, waxy, or other protective materials, that is, biodegradable synthetic polymers and modified natural products. [24]

Morphological Classification
Microspheres are characteristically free-flowing, solid spherical particles with diameters spanning from 1000 (micrometer scale). Fabricated from natural proteins (e.g., albumin, gelatin) or biocompatible synthetic polymers (e.g., PLGA), these biodegradable entities are broadly categorized into two distinct structural types based on how the active pharmaceutical ingredient (API) is distributed:

- Microcapsules (Reservoir Systems): Systems where a distinct core of the therapeutic agent is centrally entrapped and completely surrounded by an independent, continuous polymer wall or shell.
- Micromatrices (Matrix Systems): Systems where the active drug substance is homogeneously dispersed or dissolved throughout the entire interconnected polymeric matrix network.

Controlled Release Kinetics

Solid biodegradable microspheres are highly valued in pharmaceutical formulation for their ability to achieve controlled and sustained drug release. When introduced into a biological system, the drug is liberated through a combination of polymer matrix swelling, water diffusion, and gradual biodegradation (hydrolysis or enzymatic cleavage) of the polymer chains. This steady release profile maintains therapeutic drug concentrations in the body over extended periods—ranging from days to months—thereby reducing dosing frequency and minimizing side effects. [25]

Transfersomes

Transfersomes are specially optimized particles or vesicles that can respond to an external stress by rapid and energetically inexpensive, shape transformations. The development of novel approaches such as transfersomes have immensely contributed in overcoming problem faced by transdermal drug delivery such as unable to transport larger molecules, penetration through the stratum corneum is the rate limiting step, physicochemical properties of drugs hinder their own transport through skin.

These elastic vesicles can squeeze themselves through skin pores many times smaller than their own size and can transport larger molecules. Transfersomes are applied in a nonoccluded method to the skin, which permeate through the stratum corneum lipid lamellar regions as a result of the hydration or osmotic force in the skin. It can be applicable as drug carriers for a orbit of small molecules, peptides, proteins and herbal elements.

Here are key features of transferosomes:

1. **Composition:** Transferosomes are typically composed of a lipid bilayer (similar to liposomes) but have special additives like surfactants or edge activators that make them more flexible and elastic. These additives allow the vesicles to deform and squeeze through the tight junctions of the skin, which is a major barrier to drug absorption.
2. **Deformability:** One of the defining features of transferosomes is their high deformability. This allows them to "squeeze" through the skin's layers, even through pores or areas where the skin's permeability is restricted. This makes them much more effective at carrying drugs across the skin barrier compared to traditional liposomes.
3. **Drug Delivery:** Transferosomes are primarily used for transdermal drug delivery. This means they can deliver drugs directly through the skin into the bloodstream, bypassing the digestive system and avoiding the first-pass metabolism in the liver. This makes them ideal for drugs that need to have controlled release over time or drugs that would otherwise be broken down in the gastrointestinal system. [26]

Elastic Vesicle Dynamics

Transfersomes represent a highly specialized, optimized class of ultra-deformable, elastic liposomal vesicles. Developed to overcome the formidable barrier properties of the skin, a transfersome can dynamically respond to external mechanical stress by undergoing rapid, energetically inexpensive shape transformations.

Traditional transdermal delivery is severely restricted by the stratum corneum (the outermost, cornified layer of skin), which naturally prevents the passive diffusion of molecules larger than approximately 500. Transfersomes solve this limitation through their unique structural elasticity, allowing them to self-optimize and squeeze through intact skin pores that are many times smaller than the vesicle's original diameter without rupturing. [26]

Key Structural and Functional Features

- **Composition and Membrane Fluidity:** While standard liposomes consist purely of phospholipids, transfersomes are uniquely formulated by combining a phospholipid bilayer with specialized edge activators or surfactants (such as sodium cholate, Tween 80, or Span 80). These surfactants destabilize the lipid bilayer just enough to confer extreme flexibility and high curvature potential without causing vesicle lysis.
- **Osmotic Driving Force:** Transfersomes are applied to the skin under non-occluded (uncovered) conditions. This sets up a transdermal hydration gradient: the relative dryness of the skin surface compared to the moisture-rich deeper viable tissues creates an osmotic force. The transfersome utilizes this gradient, pulling itself and its therapeutic cargo through the lipid lamellar regions of the stratum corneum.
- **Therapeutic Versatility:** This platform acts as an efficient carrier for a wide spectral orbit of molecules, successfully transporting small molecular weight drugs, fragile peptides, bulky proteins, and complex hydrophobic or hydrophilic herbal extracts directly across the cutaneous barrier. [27]

Proniosomes

Proniosomes represent a significant technological advancement over conventional vesicular carriers. They are formulated as dry, free-flowing, granular product networks coated with non-ionic surfactants that can be instantly rehydrated to form classic niosomes (non-ionic surfactant vesicles) immediately prior to administration.

While standard liquid niosomal suspensions suffer from physical instability during storage—frequently experiencing drug leakage, vesicle aggregation, fusion, and chemical hydrolysis of the lipids proniosomes overcome these bottlenecks by maintaining the delivery system in a dry, solid-state condition until use. [28]

Composition and Formulation Dynamics

The structural matrix of a proniosome typically comprises three primary components:

- Non-ionic Surfactants: These form the core bilayer structure upon hydration. Commonly used surfactants include alkyl ethers or esters (e.g., Span 20, 40, 60, or Tween 20, 80), chosen for their chemical stability, low toxicity, and precise Hydrophilic-Lipophilic Balance (HLB) values.
- Membrane Stabilizers: Cholesterol or lecithin is incorporated to impart rigidity, modulate membrane fluidity, and minimize the leakage of encapsulated phytochemicals or active pharmaceutical ingredients (APIs).
- Water-Soluble Carrier Matrix: Sorbitol, mannitol, maltodextrin, or lactose crystals are utilized as solid substrate carriers. The surfactant-lipid-drug mixture is coated uniformly over these micro-granular carriers to ensure rapid dispersion during rehydration.

Mechanics of Transdermal Permeation

Proniosomes are heavily researched as non-invasive platforms for both transdermal drug delivery systems (TDDS) and the delivery of poorly soluble herbal extracts. Upon topical application, the formulation utilizes the skin's natural moisture gradient or an added aqueous vehicle to undergo in situ hydration, converting into flexible niosomal vesicles directly on the skin surface. [30]

These newly formed vesicles alter the lipid architecture of the stratum corneum through several simultaneous mechanisms:

1. Adsorption and Fusion: The vesicles fuse directly with the intercellular lipid bilayers of the skin, releasing their therapeutic cargo into deeper cutaneous layers.
2. Penetration Enhancement: The non-ionic surfactants act as chemical penetration enhancers, reversibly disrupting the highly organized skin lipids to reduce the diffusional resistance of the skin barrier.
3. Intact Vesicle Infiltration: Due to the elasticity conferred by the surfactant composition, smaller niosomal fractions can squeeze through the skin's microscopic pores via osmotic driving forces, bypassing conventional size limitations.

Key Advantages in Phytomedicine

- Superior Physical Stability: By eliminating water from the storage phase, proniosomes prevent chemical degradation, settling, and premature drug leakage, ensuring a significantly longer shelf-life compared to liposomes or liquid niosomes.
- High Encapsulation Efficiency: The high surface area of the granular carrier allows for high loading capacities of both hydrophobic and hydrophilic plant active components.
- Industrial Scalability: The production of proniosomes avoids complex, hazardous solvent-evaporation techniques often required for traditional vesicular systems, making the process highly adaptable to standard industrial pharmaceutical machinery (such as spray drying or fluidized bed coating). [28]

Transdermal drug delivery system (TDDS)

Transdermal Drug Delivery System (TDDS) are defined as self-contained, discrete dosage which is also known as patches. A transdermal patch or a skin patch is a medicated adhesion with minimal inter and intra patient variation. The main objective of transdermal drug delivery system is to deliver drugs into systemic circulation into the skin through skin at predetermined rate with minimal inter and intra patient variation.

That will improve bioavailability, more uniform plasma levels, longer duration of action resulting in a reduction in dosing frequency, reduced side effects and improved therapy due to maintenance of plasma levels up to the end of the dosing interval compared to a decline in plasma levels with conventional oral dosage forms. [30]

System Design and Patch Technology

A Transdermal Drug Delivery System (TDDS) refers to self-contained, discrete, multi-layered dosage forms commonly engineered as medicated transdermal patches. When applied to intact skin, these patches are designed to deliver a therapeutically effective amount of drug across the skin barrier and directly into the systemic circulation at a strictly controlled, predetermined rate.

Clinical Advantages Over Conventional Routes

TDDS provides a highly sophisticated alternative to traditional oral or intravenous administration by offering several distinct clinical benefits:

- Avoidance of First-Pass Metabolism: By absorbing directly into the dermal capillaries, the drug bypasses the harsh acidic environment of the gastrointestinal tract and avoids immediate hepatic first-pass elimination in the liver, drastically improving the bioavailability of sensitive drugs.
- Steady-State Plasma Concentrations: Unlike oral tablets which cause fluctuating "peak-and-valley" concentrations in the blood, transdermal patches establish a uniform, zero-order release profile. This continuous delivery maintains steady plasma levels up to the very end of the dosing interval.
- Minimized Patient Variability: TDDS formulations are engineered to achieve minimal inter-patient (between different people) and intra-patient (within the same person over time) variation, ensuring predictable therapeutic outcomes.
- Enhanced Compliance and Reduced Side Effects: Extended wear times (e.g., once-daily or once-weekly

patches) reduce dosing frequency. This convenience greatly improves patient adherence to therapy while simultaneously decreasing systemic side effects by avoiding toxic peak drug surges. [29]

Ethosomes

Ethosomes are drug-phospholipid-ethanol complexes with a soft, flexible structure that enhance the delivery of phytoconstituents from herbal extracts. The high ethanol content in ethosomes increases the permeability of the skin's lipid bilayer, promoting faster penetration of active compounds through the stratum corneum. Compared to other vesicular formulations, ethosomes offer superior stability. They enable the transdermal delivery of bioactive molecules of varying sizes, advancing the development of more effective therapeutic strategies.Ethosomes are prepared using simple, scalable techniques that do not require sophisticated equipment, making them suitable for both pilot and industrial production. The preparation process involves two main methods: "cold" and "hot" techniques (Pandey, V. et. al., 2015)

The skin is a large and easily accessible organ that offers several advantages for drug delivery, such as reduced fluctuations in plasma drug levels, avoidance of gastrointestinal issues and first-pass metabolism, and improved patient compliance. [31]

However, a major limitation is the skin's low permeability, which restricts the types of drugs that can be effectively delivered through it. The stratum corneum, the outermost layer of skin, serves as a significant barrier to most drugs, allowing only lipophilic (fat-soluble) and small molecular weight drugs to pass through easily.

Ethosomes are soft, malleable lipid vesicles made primarily of phospholipids, alcohol (ethanol or isopropyl alcohol), and water, with alcohol concentrations ranging from 20-45%. Developed by Touitou and colleagues in 1997, ethosomes are highly deformable, enabling them to effectively permeate human skin.

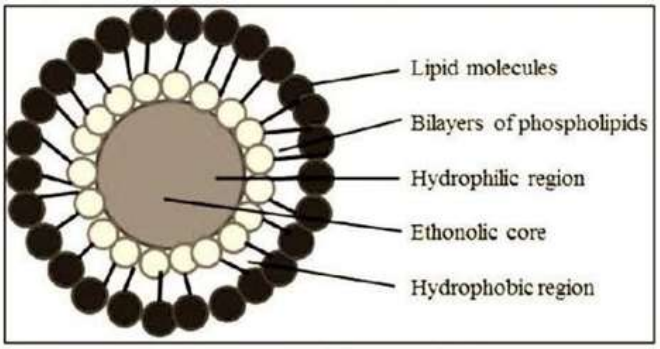


Fig.1. Structure of Ethosome

The vesicles are formed using phospholipids such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), and their derivatives, sourced from egg, soybean, or synthetic options. Alcohol concentration in ethosomes enhances vesicle flexibility and stability, while disrupting the skin's lipid bilayer to increase permeability. Glycols like propylene glycol and transcutol can further boost penetration, and cholesterol (0.1-1%) may be added to improve stability. [32]

Advantages of Ethosomal Drug Delivery: Ethosomal drug delivery offers several key benefits, particularly in comparison to other transdermal and dermal systems. These include:

1. Improved Skin Permeation: Ethosomes enhance the penetration of drugs through the skin, making them effective for transdermal delivery.
2. Delivery of Large Molecules: Ethosomes are capable of delivering larger molecules, such as peptides and proteins, which are typically challenging to administer via traditional routes.
3. Safety and Biocompatibility: The formulation of ethosomes uses non-toxic raw materials, ensuring a safer alternative for drug delivery
4. Patient Compliance: Ethosomal formulations are typically administered Improved in semisolid forms, such as gels or creams, which contribute to higher patient adherence and ease of use.
5. Non-Invasive and Passive Delivery: The ethosomal system is passive and non-invasive, offering a straightforward method for drug delivery that is ready for immediate commercialization.
6. Broad Applicability: Ethosomal drug delivery systems can be utilized across a variety of industries, including pharmaceuticals, veterinary medicine, and cosmetics.
7. Enhanced Skin Permeation: Ethosomes enhance the penetration of drugs through the skin, making them effective for both transdermal and dermal drug delivery.
8. Versatility for Different Drugs: Ethosomes provide a platform for the delivery of a wide range of drugs, including large molecules such as peptides and proteins.
9. Regulatory Approval: The components of ethosomes are approved for use in both pharmaceuticals and cosmetics, making them safe for application.
10. Low Toxicity Risk: Since the safety profiles of ethosomal components are well-documented in scientific literature, the technology poses a low risk for large-scale drug development. [34]

Limitations of Ethosomes:

1. Low Yield: The production yield of ethosomes can be low, which may limit their efficiency.
2. Stability Issues: If the shell locking process is not effective, ethosomes may break apart or aggregate when transferred into aqueous environments.

3. Product Loss: Some product loss may occur during the transfer from organic solvents to aqueous media (Nandure, H.P., et. al.,2013.)

II. METHOD OF PREPARATION OF ETHOSOMES

Ethosomes can be prepared using two main methods:

1. Hot Method:

In this approach, phospholipids are dispersed in water by heating the mixture in a water bath at 40°C until a colloidal solution is achieved. Separately, temperature. The organic phase is then added to the aqueous phase. Depending on the drug's solubility, it can be dissolved in either water or ethanol. To achieve the desired vesicle size in the ethosomal formulation, techniques such as probe sonication or extrusion may be employed.

2. Cold Method:

This method is the most commonly employed technique for preparing ethosomes. In this process, phospholipids, the drug, and other lipid components are dissolved in ethanol within a covered container at room temperature while stirring vigorously. Propylene glycol or another polyglycol is added during the stirring phase. The mixture is then heated to 30°C using a water bath. Meanwhile, water is also heated to 30°C in a separate vessel and subsequently added to the lipid-ethanol mixture. This combination is stirred for an additional 5 minutes while still covered. To achieve the desired vesicle size in the ethosomal formulation, methods such as sonication or extrusion can be utilized. (Nandure, H.P., et. al.,2013) [35]

Characterization of Ethosomes

1. **Microscopic Examination of Particle Size and Morphology**

To assess the size and shape of ethosomes, microscopic analysis was conducted. The ethosome sample was first diluted with distilled water to ensure proper observation of individual vesicles. A small drop of the diluted suspension was placed on a glass slide, covered with a cover slip, and examined under a microscope at magnifications of 15× and 45×. All measurements were repeated in triplicate, and the vesicles were found to be predominantly spherical in shape.

2. **Scanning Electron Microscopy (SEM) Analysis**

For scanning electron microscopy (SEM), the ethosomal dispersion was appropriately diluted and sonicated before placing a few drops on a grid, which was then left to dry. Once fully dried, the sample was examined using a scanning electron microscope at a magnification of 60×, with an accelerating voltage of 12 kV, maintained at 25±2°C. Images were captured for further analysis

3. **Zeta Potential Analysis**

Zeta potential refers to the measure of electrostatic repulsion or attraction between particles, which reflects their surface charge. The zeta potential of the sample was assessed using a nanoparticle analyzer (SZ-100 model) at a temperature of 25°C. Electrophoretic mobility and the average zeta potential values were directly obtained from the analyzer readings.

4. **Size Distribution**

Particle Size of Ethosomes

The size of ethosomes typically ranges from tens of nanometers to microns, depending on the formulation's composition. The particle size can be determined using techniques such as dynamic light scattering (DLS) or light microscopy in conjunction with an eyepiece micrometer, which is calibrated with a stage micrometer. To measure the vesicle size, the diameters of 150 vesicles were randomly selected and measured using the calibrated micrometers. [36]

III. MATERIALS AND METHODS

Drimia Indica:

Drimia Indica (Roxb.) Jessop (Asparagaceae) is a reputed Ayurvedic medicine for a number of therapeutic benefits, including for cardiac diseases, indigestion, asthma, dropsy, rheumatism, leprosy, and skin ailments. [11] The members of this genus are mostly deciduous and rarely evergreen, with an underground bulb.

These plants are distributed throughout Africa and Asia. Most of the species of this genus are found in southern Africa, mainly in semiarid regions with winter rainfall Various species of this genus, such as D. maritima (L.) Stearn, D. elata Jacq., D. secunda (B. Nord.) J.C. Manning & Goldblatt, and D. Indica (Roxb.) Jessop is very popular in traditional medicine in different parts of the world.[12]

Drimia Indica (Roxb.) Jessop (syn. Urginea Indica L.; Scilla Indica Roxb.) is commonly known as Indian squill, true squill, or sea onion. In Asian countries, the plant is also called ban piaz or jungli piyaz .D. Indica, mainly its bulb, is an Ayurvedic medicine popularly known as Kolkanda or Ban Palandu that is used for the preparation of various medicinal products that have applications in healthcare, and it is also used as a biocide.

Phytochemical studies on the plant have revealed that alkaloids, flavonoids, phenols, and tannins are common in all parts, while steroids are only present in bulbs. The bulbs also contain glycosides, quinones, resins, and saponins. The plant is used in Ayurveda for respiratory disorders, skin diseases, dysmenorrhea, and intestinal worms. The plant has been studied in various biological activities and has exhibited potent antidiabetic, anticancer, antimicrobial, and cardiac effects.[13][14]

Fig.2. Drimia Indica plant

Drimia Indica is a perennial herbaceous flowering plant which grows from bulbs. It has long leaves, typically 15–30 cm long by 1–2.5 cm wide, but sometimes considerably longer. The flowers, which appear in spring before the leaves, are borne in racemes on a leafless stem (scape) up to 60 cm long. The flowers are widely spaced on the raceme, which is 15–31 cm long, and are carried on stalks (pedicles) 2.5–4 cm long. Individual flowers are bell-shaped. The six pale brown tepals have white margins. The 6–7 cm long stamens have yellow anthers and filaments which are flattened at the base. Between six and nine seeds are produced in a capsule which is 1.5–1.8 cm long. Individual seeds are black and shaped like flattened ellipsoids. [16]

Classification
Class: Magnoliopsida Subclass: Liliidae Suborder: Lilianae Order: Asparagales Suborder: Asparagineae
Family: Hyacinthaceae/Asparagaceae
Genus: Drimia
Species: Indica

IV. ANTIFUNGAL ACTIVITY

To date, antibiotic therapy is still regarded as an essential treatment of secondary infections, despite the rise in microbial resistance, hence the evolution of chronic diseases. A number of studies have reported antimicrobial properties such as antibacterial, antifungal and antiviral properties of the genera Drimia in both in vitro and in vivo models. In addition, reports of the antimicrobial activities of some famous Drimia spp. are well documented; however, the antimicrobial activities of other species are limited. Of the 40 medicinal plants tested against 11 strains of bacteria, D. Indica was graded as successful against bacterial strains.

There was also a record of potent activity of D. Indica against other bacterial strains, especially Bacillus megaterium and Neisseria gonorrhoeae D. sanguinea bulbs displayed significant anti-Staphylococcus aureus activity. It was found that D. altissima had no activity against Listeria monocytogenes. No significant antiviral activity from the extract of D. maritima was reported.

Among the active compounds with antimicrobial properties are the homo iso flavanone compound from D. delagoensis and scillarenin from D. maritima. Baskaran et al. reported that D. robusta showed significant antibacterial activity. Furthermore, the utmost concentration (19.68 µg mg−1 DW) of proscillaridin A was reported in the roots of ex vitro plants.[18]

A study conducted on D. Indica displayed antifungal effects. The minimum inhibitory concentrations (MICs) ranged from 1.36 to 1.38 mg for antifungal effects. Various bioactive compounds such as salicylic acid, quercetin, coumarins, kaempferol, luteolin and apigenin were isolated from D. maritima.

Pandey and Gupta extracted the metabolites of Urginea Indica (D. Indica) from the roots, stems and leaves using polar (aqueous, methanol), dipolar (acetone) and nonpolar (chloroform) solvents.The plant extracted gives activity against two fungi, Aspergillus niger and Candida albicans. Fungi A. niger and C. albicans were inhibited by root acetone extract. Furthermore, the phytochemical analysis showed major compounds such as alkaloids, tannins, quinones, saponins, flavonoids, glycosides, phytosterols and resins.[19]

The Clinical Imperative
Despite the critical global surge in multidrug-resistant (MDR) microbial strains and the subsequent progression of acute infections into difficult-to-treat chronic diseases, orthodox antibiotic therapy remains the primary defensive countermeasure against secondary microbial infections.

This escalating resistance crisis has intensified the pharmaceutical exploration of botanical alternatives. Consequently, a robust body of literature has validated the wide-spectrum antimicrobial—specifically antibacterial, antifungal, and antiviral—potency of the genus Drimia across various in vitro and in vivo experimental models.

Comparative Species-Specific Antibacterial Efficacy
While the therapeutic profiles of several high-profile Drimia species are thoroughly documented, smaller sub-species continue to undergo baseline validation. In a comprehensive comparative screening evaluating 40 distinct medicinal flora against 11 pathogenic bacterial strains, Drimia indica (Indian Squill) was classified as highly successful, demonstrating superior bactericidal efficacy.

The broader literature highlights distinct antibacterial phenotypes across the genus:

- **Targeted Susceptibility:** D. indica possesses potent, targeted activity against challenging bacterial isolates, most notably Bacillus megaterium and Neisseria gonorrhoeae.
- **Gram-Positive Inhibition**: Crude extracts derived from Drimia sanguinea bulbs exhibit pronounced inhibitory action against Staphylococcus aureus. Similarly, Drimia robusta has been verified as a highly effective antibacterial agent.
- **Selectivity and Spectrum Limits**: Biological screens show clear boundaries in efficacy; for instance, Drimia altissima exhibits no detectable inhibitory activity against Listeria monocytogenes, and Drimia maritima extracts show negligible antiviral performance.

Phytochemical Matrix and Secondary Metabolites
The antimicrobial properties of the genus Drimia are directly driven by a complex matrix of secondary metabolites. Advanced phytochemical isolation has linked specific fractions to targeted therapeutic outcomes:

Species

Table No.1: Phytochemical Markers and Validated Antimicrobial Profiles of Prominent Drimia Species.

Species	Isolated Bioactive Marker	Validated Pharmacological Action
Drimia delagoensis	Homoisoflavanone compounds	Broad-spectrum antimicrobial disruption.
Drimia maritima	Scillarenin, Salicylic acid, Quercetin, Coumarins, Kaempferol, Luteolin, Apigenin	Synergistic anti-inflammatory, antioxidant, and membrane-disrupting activities.
Drimia robusta/ Ex Vitro Roots	Proscillaridin A (Peak concentration:19.68mg Dry Weight)	Highpotency cardiac glycoside with secondary antimicrobial traits.

Antifungal Dynamism and Solvent Extraction Profiles
Focusing heavily on Drimia indica, dedicated mycological assays confirm its role as a powerful antifungal agent. Quantitative testing reveals that the Minimum Inhibitory Concentrations (MICs) required to halt fungal proliferation fall within a narrow, reproducible window of 1.38.

Extensive solvent-fractionation studies (e.g., Pandey and Gupta) mapped the distribution of these active metabolites across the roots, stems, and leaves of D. indica using a polarity gradient:

- **Solvent Spectrum:** Polar systems (aqueous, methanol), dipolar agents (acetone), and nonpolar media (chloroform).
- **Fungal Target Outcomes:** The resulting extracts achieved definitive growth inhibition against two medically significant fungal pathogens: Aspergillus niger and Candida albicans. Specifically, the root acetone extract demonstrated the highest potency in halting the replication of both A. niger and C. albicans.

V. DRUG AND POLYMER PROFILE

DRUG PROFILE
Drug name: 29 kDa Glycoprotein (DrimiaIndica)
Synonyms:1,3-Benzodioxole-5-carboxylicacid, Benzo[d][1,3]dioxole-5-carboxylic acid, Heliotropic acid
Molecular Weight: 29 kilodaltons (kDa)
Melting Point: 460C

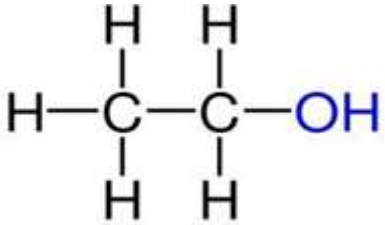
Solubility: Slightly soluble in water, ethanol,methanol
Storage: Recommended to be stored at room temperature in a cool, dark place

Description:
A 29kDa glycoprotein can be referred to as a protein that has a molecular weight of 29 kilodalton (kDa) and is also a glycoprotein, meaning it has carbohydrate molecules attached to its amino acid chain.

POLYMER PROFILE:

1. Ethanol:
Synonyms: Ethyl alcohol, grain alcohol, or alcohol Chemical Name: Ethanol Molecular Formula: C2H5OH Molecular weight: 46.07 g/mol

Chemical Structure:



Description: ethanol is a reasonable, discouraging, dubious fluid with a brand name smell. When debilitated, it is fairly sweet, yet connected with liquor has areas of strength for basic for central for a, taste.

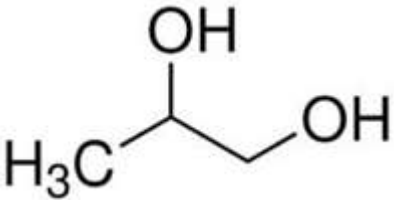
Solubility: Miscible with water & with various standard solvents, including acidic harming, CCl4, chloroform, diethyl ether, EG.
Boiling Point: 78.37 °C Acid value: 34.5 mgKOH/g HLB value: 9-20 pH: 7.33
Surface tension of 1% aqueous solution (mN/m): 2.189 X 10-2 N m-1
Viscosity at 25°C (mPas): 4.405 mPa

Density: 789 kg/m³
Application in Pharmaceutical Formulation or Technology Some medicines utilize ethanol as a solvent to disperse the active ingredient, and herbal medicines employ it as an extraction solvent.

Stability and storage Conditions: put away in fixed polyethylene bottles at 4~ & at room temperature.
Safety: Put on gloves, a face shield, and eye protection (as needed to prevent skin and eye contact with liquid). After handling, carefully wash your hands or any skin that came into contact with liquid. Avoid eating, drinking, and smoking while taking this product. Avoid inhaling fumes.

2. Propylene Glycol:
Synonyms: propylene glycol ,1,2-propanediol, propane-1,2-diol
Molecular Formula: C3H8O2
Molecular Weight: 76.09 g/mol

Melting Point: 59°C
pH: 9.3-10.5
Chemical Structure:



Color: Colorless viscous liquid
Odor: Practically odourless
Taste: Practically tasteless

IUPAC Name: propane-1,2-diol
Viscosity: 0.581 cP at 20 °C
Surface Tension: 40.1 dynes/cm at 25 °C

Description:
Propylene glycol is a synthetic liquid substance that absorbs water. Propylene glycol is also used to make polyester compounds, and as a base for deicing solutions. Propylene glycol is used by the chemical, food, and pharmaceutical industries as an antifreeze when leakage might lead to contact with food. The Food and Drug Administration (FDA) has classified propylene glycol as an additive that is "generally recognized as safe" for use in food. It is used to absorb extra water and maintain moisture in certain medicines, cosmetics, or food products. It is a solvent for food colors and flavors, and in the paint and plastics industries. Propylene glycol is also used to create artificial smoke or fog used in fire-fighting training and in theatrical productions. Other names for propylene glycol are 1,2-dihydroxypropane, 1,2-propanediol, methyl glycol, and trimethyl glycol. Propylene glycol is clear, colorless, slightly syrupy liquid at room temperature.

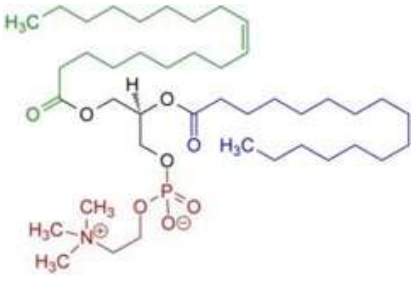
3. Soya Lecithin
Synonyms: Soybean phospholipid, Lecithin
Chemical Name: [(2R)-3-hexadecanoyloxy-2-[(9Z,12Z)-octadeca-9,12-dienoyl] oxypropyl] 2-(trimethylazaniumyl)ethyl phosphate
Molecular
Formula: C42H80NO8P Molecular weight: 758.1 g/mol
Appearance: A viscous, amber-colored liquid Taste: Bland
Melting Point: 236-237 °C

Solubility: Insoluble in water, Soluble in chloroform, ether, petroleum ether, mineral oils and fatty acids. Insoluble in acetone; practically insoluble in cold vegetable and animal oils, Soluble in about 12 parts cold absolute alcohol.

Density: 1.0305 at 24 °C/4 °C
Stability / Shelf Life: Stable under recommended storage conditions.
pH: 6.6
Odor: Odourless or slight nutlike odour; faint fatty odour
Iodine value: 95
Saponification value: 196

Fig.3. Soya lecithin

Chemical Structure:



Description
1-hexadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phosphocholine is a phosphatidylcholine 34:2 in which the 1- and 2-acyl groups are specified as hexadecanoyl (palmitoyl) and 9Z,12Z-octadecadienoyl (linoleoyl)

respectively. It is a phosphatidylcholine 34:2 and a 1-acyl-2-linoleoyl-sn-glycero-3-phosphocholine betain
Lecithin is a phospholipid with a polar cholinefound in phosphoester linkage to diacylglycerol.

Applications:
Food, cosmetics, pharmaceuticals, nutraceuticals, and healthcare

Application in Pharmaceutical Formulation or Technology

- Soya phospholipids support the structure of the membrane and maintain the permeability barrier.
- Acetylcholine, which is present in the brain, undergoes a metabolic reaction thanks to soy phosphatidyl choline.
- PC is a component of the complicated bile fluids that emulsify the body's naturally occurring fat.
- Uses: An emulsifier, stabilizer, dispersant, wetting agent, antioxidant, binding agent, viscosity reducer, anti-spattering agent, and release agent

EXTRACTION OF DRIMIA INDICA

15g powdered material was extracted with 150 ml of different solvents according to their increasing polarity successively for 8-10 hours in the soxhlet apparatus (Tempo) at a temperature not exceeding the boiling point of the respective solvents. After extraction excess solvent was removed by distillation and the concentrated extracts so obtained were further dried in incubator at 40◦C. The percentage yield and other physical properties were recorded. The residual extracts after drying were dissolved in 50% dimethyl sulphoxide and stored in refrigerator at 4o C in small and sterile glass tubes.

Principles of Successive Solvent Extraction: Extracting the plant material with solvents in order of increasing polarity (typically starting with non-polar solvents like petroleum ether or hexane, moving to mid-polar solvents like chloroform or ethyl acetate, and finishing with highly polar solvents like methanol or water) ensures a comprehensive chemical profile. This sequential separation systematically isolates lipophilic compounds (fats, waxes, sterols) first, followed by mid-polar compounds, and finally polar bioactives (flavonoids, tannins, glycosides), preventing complex chemical overlap.

Soxhlet Mechanics and Exhaustive Extraction: The Soxhlet setup provides a continuous, automated cycle of hot solvent distillation and siphoning. As the solvent boils in the distillation flask, its vapors travel up into a condenser, drop back down onto the powdered Drimia indica inside the extraction thimble, and pool until the siphon tube triggers a flush back into the main flask. This continuous rinsing loop ensures maximum extraction efficiency using a relatively low total volume of solvent ($1:10$ solid-to-solvent ratio).

Thermal Protection of Bioactives: Maintaining the operating temperature at or slightly below the boiling point of each respective solvent is critical to preserve the integrity of the target molecules. The primary active constituents in Drimia indica particularly bufadienolides and cardiac glycosides are thermosensitive. Keeping the extraction temperature regulated and performing the final concentration drying in a mild 40°C incubator actively prevents thermal degradation and hydrolysis of these delicate chemical rings.

Solubilization and Storage Dynamics: Dimethyl sulfoxide (DMSO) at a 50% concentration acts as an excellent universal co-solvent. It easily dissolves both the highly lipophilic and highly hydrophilic constituents present in the dried crude extract residues. Storing the final solution in sterile glass containers at 4°C slows down molecular kinetic energy, effectively preventing unintended chemical oxidation, mold growth, or precipitate formation before formulation into ethosomes begins.

VI. QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemical analysis of the extracts of root, stem and leaf of Urginea Indica were performed.

1) Alkaloids:
Alkaloids are defined as a group of naturally occurring chemical compounds that mostly contain basic nitrogen atoms. Alkaloids are produced by a large variety of organisms including bacteria,fungi, plants, and animals. (21)

Test for Alkaloids

- **Mayer's Test:** To 1ml of extract add 1ml of Mayer's reagent (Potassium mercuric iodide solution) whitish yellow or cream color precipitate Indicated the presence of alkaloids.
- **Hager's Test**: To 1ml of the extract, add 1ml of Hager's reagent (Saturated aqueous solution of picric acid). A yellow color precipitate Indicates the presence of alkaloids.
- **Wagner's Test**: To 1 ml of the extract, add 1 ml of Wagner's reagent (Iodine in potassium iodide solution). Formation of reddish brown precipitate Indicates the presence of alkaloids.

2) Flavonoids:
Flavonoids, a group of natural substances with variable phenolic structures, are found in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. These natural products are well known for their beneficial effects on health and efforts are being made to isolate the ingredients so called flavonoids.
Test for Flavonoids

- **Alkaline reagent Test:** Test solution when treated with NaOH solution shows increase in intensity of yellow colour, which becomes colorless on addition of few drops of dilute acid.
- **Lead acetate solution Test:** Test solution with few drops of lead acetate solution (10%) gives yellow precipitate.

3) Saponins:

Saponins are chemical compounds found in plants and marine animals that are known for their soap-like properties and ability to produce froth in water. They are a heterogeneous group of sterol glycosides and triterpene glycosides that are composed of a hydrophobic aglycone and hydrophilic sugar moieties.

Test for Saponins

- **Foam Test:** The extract was shaken vigorously with water. Persistent foam was observed.

4) Tannins:

Tannins are the polyphenolic, complex, organic and non-nitrogenous compound having a higher molecular weight. They are used as a antiseptic and in git diseases like diarrhoea and also in a leather industry. (22)

Test for Tannins

- Gelatin Test: To the extract added 1% gelatin solution in water and appearance of white precipitate was observed.
- Feric chloride Test: To the extract added 5% ferric chloride solution and deep blue-black colour was observed.

5) Quinones:

The quinones are a class of organic compounds that are formally "derived from aromatic compounds [such as benzene or naphthalene] by conversion of an even number of –CH= groups into –C(=O)– groups with any necessary rearrangement of double bonds", resulting in "a fully conjugated cyclic dione structure. (23)

Test for Quinones

A few drops of 1% NaOH was mixed with the plant extract and shaken vigorously. A blue green or red colour Indicates the presence of quinones.

6) Resins:

Resins are metabolic byproducts of plants that are solid or semi-solid amorphous substances with a complex chemical composition. A resin is a solid or highly viscous substance of plant or synthetic origin that is typically convertible into polymers.

‣Test for Resins

To the extract in a test tube, 5ml of the 1% copper acetate solution was added and the resulting solution was shaken vigorously and allowed to separate. The separation of a green colored solution was considered as positive for resins.

Chemical Principle of the Copper Acetate Test: Resins are complex, amorphous mixtures of organic compounds that typically contain high concentrations of resin acids (such as diterpene or triterpene acids). When copper acetate is introduced to the extract, these free carboxylic acid groups undergo a metathesis reaction, binding with the divalent copper ions to form lipophilic copper salts or complexes of resin acids. Mechanism of Color Formation: The resulting green color is highly characteristic of copper-resin complexes (often called copper resinates). This specific coloration occurs due to the coordination geometry established between the copper ion and the organic resin molecules, which alters the d-orbital electron configurations of the copper and causes it to absorb light in the red-orange spectrum, reflecting a distinct green or bluish-green hue.

The Role of Phase Separation: Shaking the solution vigorously maximizes the surface contact between the aqueous copper acetate reagent and the organic phytoconstituents. Because copper resinates are inherently lipophilic and hydrophobic, they do not remain dissolved in a purely polar environment. Allowing the test tube to rest permits the mixture to separate into distinct layers, concentrating the green-colored copper complexes into the organic phase, making the positive result easily visible.

Table No.4: Results of identification test for Drimia Indica

Sr. No.	Test	Result
1	**Test For Alkaloids**	
	A) Mayer's Test	Pass
	B)) Hager's Test	Pass
	C)) Wagner's Test	Pass
2	**Test For Flavonoids**	
	A) Alkaline Reagent Test	Pass
	B) Lead Acetate Solution Test	Pass
3	**Test For Saponins**	
	A) Foam Test	Fail
4	**Test For Tannins**	
	A) Gelatin Test	Fail
	B) Feric Chloride Test	Fail
5	**Test For Quinones**	Pass
6	**Test For Resins**	Pass

VII. FORMULATION OF ETHOSOMES

Ethosomes were prepared as reported by Touitou et al. (2000)

1. Weighing of Ingredients:

- Accurately weigh the required amount of phospholipid (lecithin) and 29kDa glycoprotein(drug).

- Measure the specified volume of ethanol, propylene glycol, and distilled water as per the design table.

2. **Preparation of Ethosomal Suspension:**

- Dissolve the phospholipid in the required amount of ethanol under gentle heating (40-50°C) while stirring continuously to form a clear solution.
- Add propylene glycol to the solution and stir for further 10-15 minutes to ensure uniform mixing.
- In a separate container, 29kDa glycoprotein(drug) is dissolved in a small volume of ethanol (if necessary) and added to the phospholipid-ethanol mixture.
- Mix thoroughly to achieve a homogenous solution.

3. Thin-Film Hydration:

- The ethanol-solvent mixture containing phospholipid and 29kDa glycoprotein(drug) is placed in a rotary evaporator.
- The solvent is removed under reduced pressure at 40°C to form a thin lipid film on the wall of the rotary evaporator flask.
- After complete solvent evaporation, the thin lipid film is hydrated with the remaining volume of distilled water at room temperature or slightly higher (typically around 40°C).
- Stir gently for 30 minutes to allow full hydration of the lipid film.

4. **Size Reduction (Sonication):**

- After hydration, the suspension is sonicated using a probe sonicator or bath sonicator at an appropriate power setting for 5–10 minutes to reduce the particle size and form nanosized vesicles.
- The particle size can be further optimized based on the design and requirements.

Table No 4: Formulation table

Sr.No.	Ingredients	F1	F2	F3
1.	29kDa glycoprotein(g)	0.2 g	0.2 g	0.2 g
2.	Phospholipid Quantity(g)	0.2 g	0.2 g	0.2 g
3.	Propylene glycol(g)	1 mL	1 mL	1 mL
4.	Ethanol Quantity(mL)	4 mL	5 mL	6 mL
5.	Distilled water(mL)	14.8 mL	14 mL	13 mL
6.	Total Volume(mL)	20 mL	20 mL	20 mL

VIII. EVALUATION PARAMETERS OF ETHOSOMES

1. Physical appearance
2. Zeta potential
3. Spreadability
4. pH
5. Washability

1. **Physical appearance:** The ethosomal gel formulations were evaluated for their organoleptic characteristics, including color, odor, texture upon application.
2. **Zeta potential:** Zeta potential refers to the measure of electrostatic repulsion or attraction between particles, which reflects their surface charge. The zeta potential of the sample was assessed using a nanoparticle analyzer (SZ-100 model) at a temperature of 25°C. Electrophoretic mobility and the average zeta potential values were directly obtained from the analyzer readings.
3. **Spreadability:** Spread ability was assessed using a modified wooden block and glass slide setup. A specified amount of gel (0.5 g) was placed on a fixed glass slide, which had a 1 cm diameter circle. A movable pan with an attached glass slide was then placed over the fixed slide, sandwiching the gel between the two for 5 minutes. The increase in the diameter of the gel due to spreading was measured, and spreadability was calculated using the following formula:

$$S=M/T$$

Where, S is the Spreadability in g/s, M is the mass in grams and T is the time in seconds.

4. **pH:** Its pH was recorded by using a pH paper
5. **Washability:** The washability of the gel was tested by applying a small amount to the skin and then rinsing with water to determine if the gel was fully remo

To thoroughly characterize an ethosomal system, the evaluation is typically divided into two phases: Characterization of the Ethosomal Suspension (the vesicles themselves) and Characterization of the Ethosomal Gel (the final topical dosage form). [31]

I. Characterization of Ethosomal Suspensions (Vesicles).

Beyond physical appearance, the microscopic and electrochemical properties of the vesicles dictate their stability and skin-penetration capabilities.

1. Vesicle Size, Polydispersity Index (PDI), and Morphology

While physical appearance gives a macroscopic view, microscopic evaluation is crucial for nano-carriers.

- **Vesicle Size and PDI:** Measured using Dynamic Light Scattering (DLS) or Photon Correlation Spectroscopy at 25°C. A narrow PDI (closer to 0) indicates a homogenous size distribution, which is vital for uniform skin penetration.
- **Morphology:** The spherical shape and lamellar nature of ethosomes are confirmed using Transmission Electron Microscopy (TEM) or Scanning Electron Microscopy (SEM).

2. Zeta Potential

- **Significance:** Zeta potential quantifies the net surface charge of the ethosomes, which dictates the physical stability of the suspension. High positive or negative values (generally outside the range of induce strong electrostatic repulsion between adjacent vesicles, preventing aggregation or coalescence over time.
- **Measurement:** Assessed via electrophoretic mobility using a nanoparticle analyzer (e.g., Malvern Zetasizer or SZ-100 model) at a controlled temperature of 25°C. [24]

3. Entrapment Efficiency (EE%)

- **Significance:** This determines the percentage of the active pharmaceutical ingredient (API) successfully entrapped within the lipophilic bilayers or the aqueous core of the ethosomes.
- **Measurement:** The unentrapped drug is separated from the ethosomes using ultracentrifugation or dialysis. The amount of free drug in the supernatant is analyzed using UV-Visible Spectroscopy or HPLC. The EE% is calculated using the formula:

Characterization of Ethosomal Gel Formulations.

When ethosomes are incorporated into a gel base (like Carbopol) for topical application, the final product must undergo rigorous rheological and performance testing.

4. Organoleptic Evaluation (Physical Appearance)

- **Methodology:** The developed ethosomal gels undergo visual and sensory inspection. They are evaluated for color (clarity vs. opacity), distinctive odor (often influenced by the ethanol content), and tactile texture (smoothness, grittiness, or stickiness upon skin application).

5. Rheological Profile and Viscosity

- **Significance:** Viscosity influences how easily the gel is extruded from a tube and how it behaves under shear stress (rubbing on the skin). Ethosomal gels ideally exhibit pseudoplastic (shear-thinning) behavior, meaning they become less viscous and flow easily when rubbed.
- **Measurement:** Measured using a Brookfield viscometer (or a rotational rheometer) with appropriate spindle selection at varying shear rates. [28]

6. Spreadability

- Significance: This parameter measures the ease with which the gel spreads over the affected skin surface, ensuring uniform drug dosing.
- Measurement: Evaluated using a specialized parallel-plate or wooden block setup. A designated mass of gel (e.g., 0.5 g) is centered on a fixed glass slide within a pre-marked boundary. A movable upper glass slide is lowered onto it, and a specific weight is applied for a set duration (e.g., 5 minutes) to mimic spreading pressure.
- Calculation: The expanded diameter is recorded, and spreadability is quantified using the kinetics of mass moving over time:

Where:

1. S = Spreadability
2. M = Weight tied to the upper slide / weight applied
3. L = Length of the glass slide
4. T = Time taken to separate the slides completely

(Note: If evaluating purely by area expansion, the increase in surface diameter is directly reported).

7. Extrudability Study

1. Significance: Measures the force required to expel the gel from a commercial tube.
2. Method: The formulation is filled into a collapsible aluminum or plastic tube. A fixed weight or compressive force is applied to the crimped end, and the percentage of gel extruded is calculated. An empirical rating is assigned (Excellent, Good, or Fair).

8. pH Determination

- Significance: Topical formulations must align closely with human skin chemistry to avoid erythema, inflammation, or tissue irritation.
- Measurement: While pH paper gives a quick estimate, precise quantification requires a calibrated digital pH meter. The electrode is immersed directly into a 10% w/v aqueous dispersion of the ethosomal gel, ensuring the reading stabilizes between the ideal skin-compatible range of 5.0 to 6.5.

9. Washability

- **Significance:** Ensures patient compliance. A topical formulation should remain on the skin long enough to deliver the drug but be easily removable without vigorous scrubbing.
- **Methodology:** A standardized thin layer of the gel is applied to an outlined area on the volar forearm or a synthetic skin substrate. After a brief drying interval, it is exposed to a gentle stream of distilled water. The ease of removal and the presence of any greasy or hydrophobic residue are visually and texturally audited. [27]

III. Performance & Stability Testing (Advanced Parameters).

In Vitro Drug Release and Ex Vivo Skin Permeation

- Method: Conducted using a Franz Diffusion Cell apparatus equipped with either a synthetic membrane (for release) or excised animal/human skin (for permeation). The ethosomal gel is placed in the donor compartment, and samples are drawn from the receptor medium at designated

intervals to map the cumulative percentage of drug delivered across the barrier over time.

Accelerated Stability Studies

- **Method:** The ethosomal gel is stored in its final packaging under stressed environmental conditions according to ICH guidelines. Over a period of 1 to 6 months, samples are periodically withdrawn to check for phase separation, vesicle leakage, drug degradation, or drops in viscosity. [26]

IX. RESULTS AND DISCUSSION

Table no.5: Result of evaluation parameters

Sr.no.	Formulation	Colour	Appearance	Spreadability (g.cm/sec)	pH	Washability
1.	F1	Creamy	Homegeneous	35.07+0.86	5.6	Easily washable
2.	F2	Creamy	Homegeneous	33.72+0.52	5.8	Easily washable
3.	F3	Creamy	Homegeneous	34.62+0.62	5.5	Easily washable

Organoleptic Assessment and Physical Uniformity: The creamy color and completely homogeneous appearance observed across all three batches (F1, F2, and F3) indicate successful vesicular integration and uniform dispersion of the Drimia indica ethosomes within the formulation base. The absence of phase separation, visible graininess, or structural separation confirms that the surfactant and lipid ratios chosen are structurally stable and provide excellent physical consistency.

Rheological Dynamics and Spreadability: Spreadability is a key factor in determining how easily a topical formulation applies to the skin and how comfortably a patient can use it. The values ranging from 0.52 g for F2 0.86 g for F1 demonstrate an ideal balance of flow behavior. This range ensures that the product spreads smoothly across an inflamed or painful area under mild skin pressure, without running off the skin or requiring rough rubbing during application.

Biocompatibility and pH Optimization: The recorded pH values of the formulations (5.5 for F3, 5.6 for F1, and 5.8 for F2) line up perfectly with the natural acid mantle of healthy human skin, which typically ranges from 4.5 to 6.0. Maintaining the formulation within this narrow, weakly acidic range is critical to prevent localized skin irritation, redness, or disruption of the protective epidermal barrier, confirming that the ethosomal matrix is safe for long-term transdermal application.

Washability and Patient Compliance: The "easily washable" status verified for all three batches indicates that the base formulation can be cleanly removed with water without leaving an oily, sticky, or occlusive residue on the skin surface. This characteristic significantly improves patient compliance, making the topical treatment highly practical for day-to-day use over extended periods. [35]

X. CONCLUSION

In this research, an innovative ethosomal system loaded with a plant-derived extract was successfully developed and tested for its antifungal capabilities. Due to their high elasticity and ability to temporarily fluidize skin lipids, the engineered ethosomes efficiently breached the stratum corneum barrier, ensuring that the active botanical constituents reached deeper epidermal layers.

The system also exhibited a controlled release pattern, which helps maintain a steady therapeutic effect over time while minimizing how often a patient needs to reapply the treatment. By leveraging the multi-targeted strengths of natural compounds, this formulation offers a highly biocompatible substitute for traditional synthetic drugs, potentially addressing the urgent clinical issues of drug-resistant fungi and topical irritation.

While advancing this technology toward commercial use demands subsequent industrial scale-up, stability trials, and in vivo clinical assessments, this study confirms that ethosomes serve as an exceptional vehicle for optimizing herbal remedies against superficial fungal pathologies.

Vesicular Deformability and Elastic Penetration Mechanisms: The successful breach of the stratum corneum highlights the specific mechanical performance of ethosomal carriers. Because these structures combine phospholipids with high levels of ethanol, the vesicle membranes possess an ultra-deformable, elastic structure. This allows them to dynamically change shape and slide intact through microscopic gaps in the skin lipid matrix, delivering the raw plant compounds directly to deep tissue layers where fungi typically take root.

Pharmacokinetic Benefits of Sustained-Release Pools: The controlled release pattern observed in this study points to the creation of a local drug reservoir within the deep layers of the skin. Instead of a rapid, short-lived burst that quickly fades, the ethosomes slowly leak their active botanical compounds over an extended period. This steady release keeps local drug concentrations consistently above the Minimum Inhibitory Concentration (MIC) needed to kill fungi, improving therapeutic success while reducing the daily application burden on the patient.

Mitigating Antifungal Resistance via Multi-Target Action: Using a whole plant extract inside the ethosomes offers a distinct advantage over single-compound synthetic antifungals (like azoles). Synthetic drugs target only one specific cellular pathway, making it easy for fungi to mutate and develop resistance. In contrast, the diverse phytochemicals inside this botanical extract attack the fungal cells through multiple pathways simultaneously disrupting cell membranes, inducing oxidative stress, and blocking enzyme production all at once which heavily cuts down the chance of the fungi developing drug resistance.

Translational Pipeline and Scaling Considerations: To move this ethosomal system from successful laboratory testing into a real-world commercial product, a clear industrial scale-up pathway must be established. Future steps must focus on evaluating long-term physical stability specifically monitoring the formulation to ensure the volatile ethanol does not evaporate and cause the nanocarriers to leak or collapse over time. Additionally, moving into in vivo animal models and human clinical trials will be essential to fully confirm the real-world safety, skin tolerability, and healing success of the product on living tissue.

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