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Review Article

Transferosome Based Delivery of Phytoconstituents in Superficial Fungal Infections: A Comprehensive Review

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Abstract

Superficial fungal infections, such as dermatophytosis, candidiasis, and pityriasis versicolor, are common worldwide and often affect the quality of life. Their incidence is increasing due to factors such as climate change, global travel, and the extensive use of immunosuppressive therapies. Conventional antifungal agents, including azoles and allylamines, face limitations such as drug resistance, poor skin penetration, and adverse effects. Plant-derived phytoconstituents contain several bioactive compounds with promising antifungal activity; however, their topical use is restricted because of their low permeability through the stratum corneum. Transferosomes, highly flexible lipid vesicles, offer an effective strategy for enhancing the skin delivery of phytoconstituents. This review explains the basic principles of transferosomes technology, discusses the antifungal activities of various plant-derived phytoconstituents, and reviews preparation methods, characterization techniques, and findings from in vitro and in vivo studies. This study aimed to highlight the potential of transferosomes as a novel and efficient approach for delivering phytoconstituents to treat superficial fungal diseases.

Keywords: antifungal, drug delivery, fungal infections, phytoconstituents, topical treatment, transferosomes

1. Introduction

Fungal infections affecting the skin, such as dermatophytosis, candidiasis, and pityriasis versicolor, are a major global health issue, impacting individuals' quality of life and leading to high healthcare expenses. These skin infections impose a heavy health burden worldwide, affecting millions of people. Although traditional antifungal treatments are generally effective, they face challenges such as drug resistance, poor skin absorption, and side effects, prompting the need to explore alternative treatment options[1,2]. Traditional antifungal medications, including azoles and allylamines, have long been the treatment cornerstone. Nonetheless, the rise of drug resistance, especially in species such as Candida auris and Trichophyton mentagrophytes, presents a significant challenge to effective management[3]. The most drug-resistant fungi include species from the Candida and Aspergillus genera. Some pathogens are found worldwide, while others are limited to specific regions[4]. Moreover, these agents typically have poor skin penetration, resulting in inadequate drug levels at the affected site, and can cause unwanted side effects, such as skin irritation and systemic harm[5]. In response to these challenges, the exploration of natural products and innovative drug delivery systems has gained significant momentum. Herbal remedies, with their diverse array of bioactive compounds, have demonstrated promising antifungal activity against a wide range of dermatophytes and yeast. Curcuma longa, Clove, Cinnamon oil, Neem, Datura metel, and Zingiber officinale have been studied as herbal sources with potent antifungal properties. offers a rich source of natural compounds with both antifungal and immune-boosting properties, suggesting that combining these approaches could lead to more effective treatment. Plant-derived antimicrobials can be used independently or in conjunction with conventional antibiotics. [6–10]. The stratum corneum presents a significant barrier to the effective topical delivery of herbal compounds to the skin. To mitigate this, ultra-deformable lipid vesicles, known as transferosomes, have been investigated. These vesicles, composed of phospholipids and edge activators, facilitate transdermal drug delivery through their enhanced flexibility and ability to interact with lipid bilayers of the skin.[11–13].

This review provides a comprehensive overview of the current state of research on herbal transferosomes for the treatment of topical fungal diseases. This review delves into the fundamental principles of transferosomes technology, explores the antifungal potential of various phytoconstituents, and critically analyzes in vitro and in vivo studies evaluating the efficacy of these novel delivery systems.

2. Common Fungal Pathogens and Their Associated Diseases

Topical fungal infections affecting the superficial layers of the skin, hair, and nails are common clinical presentations, affecting a significant proportion of the global population. Table 1 shows the diversity of topical fungal infections, highlighting the specific organisms and clinical presentations.

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Table 1: Fungal Organisms and Diseases

Disease	Responsible Organisms	Common Locations	Ref
Tinea Pedis (Athlete's Foot)	*Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*	Between toes, soles of feet	14
Tinea Cruris (Jock Itch)	*Trichophyton rubrum*, *Epidermophyton floccosum*	Groin, inner thighs	15
Tinea Corporis (Ringworm)	*Trichophyton* spp., *Microsporum* spp.	Body, face, limbs	16
Tinea Capitis (Scalp Ringworm)	*Trichophyton* spp., *Microsporum* spp.	Scalp, hair follicles	16
Onychomycosis (Nail Fungus)	*Trichophyton rubrum*, other dermatophytes, *Candida* spp.	Fingernails, toenails	17
Cutaneous Candidiasis	*Candida albicans*, other *Candida* spp.	Skin folds, diaper area, mouth	18
Malassezia Folliculitis	*Malassezia* species	Chest, back, upper arms	19
Seborrheic Dermatitis	*Malassezia* species	Scalp, face, chest	20

3. Drying of Biomass – Different drying techniques

Appropriate drying techniques help reduce moisture content, prevent microbial growth, increase shelf life, and facilitate handling and storage. However, improper drying can lead to the loss or degradation of heat-sensitive bioactive compounds. A comparative overview of commonly used drying methods is presented in Table 2

Table 2: A Comparative Analysis of Plant Drying Methods

Drying Method	Description	Advantages	Disadvantages	Ref
Sun Drying	Fresh herbs placed on ventilated racks and exposed to direct sunlight.	Simple and low cost.	Possible degradation of color, aroma, and heat-sensitive compounds; not ideal for all plants.	21,22
Shed Drying	Herbs dried in shaded areas with good ventilation.	Preserves essential oils; suitable for heat-sensitive herbs	Longer drying time.	21,22
Solar Drying	Uses solar energy, either directly or through heated air from solar collectors.	Better preservation of flavor and phytoconstituents compared to open sun drying.	Requires specialized solar drying equipment.	22,23
Hot Air Oven Drying	Uses controlled temperature, air flow, and time for uniform drying.	Controlled conditions; good for moisture removal and volatile reduction.	High temperatures (>55°C) may cause oxidation or loss of sensitive compounds.	24,25
Freeze Drying (Lyophilization)	Removes water by sublimation under vacuum after freezing the material.	Excellent preservation of phytochemicals; removes up to 95% of water.	Expensive and requires specialized equipment.	24,26,27
Microwave Drying	Uses microwave energy to rapidly heat water molecules inside plant tissue.	Very fast drying; retains essential oil components effectively.	Uneven heating may occur, especially with large batches.	21,22,28

4. Extraction methods

Selecting an appropriate extraction method is essential for obtaining antifungal phytoconstituents intended for transferosomes encapsulation. The ideal technique should prevent thermal degradation, ensure high extraction efficiency, and be compatible with the transferosomes preparation process. Several commonly used extraction methods are described below.

I. Maceration Method

Maceration is a gentle and straightforward extraction technique in which crushed plant material is soaked in a suitable solvent (menstruum) for several days at room temperature. The mixture is periodically agitated to enhance extraction. After extraction, the liquid is separated from the solid residue (marc), which is pressed to recover any remaining solvent [9]. Although maceration is easy to perform, it has limitations such as long extraction times and relatively low efficiency. However, it is particularly suitable for thermolabile compounds that may degrade at high temperatures[29]. Different solvents can extract various classes of phytochemicals—such as glycosides, alkaloids, terpenoids, and saponins—depending on their polarity.[30]. *Ane Patrícia Cacique* reported that, Catharanthus roseus extracts were optimized for total phenol content using maceration. The optimal conditions were 30 mg dry plant tissue in 50:50 ethanol-water at 50 °C for 1 hour[31].

II. Percolation Method

Percolation is generally more efficient than maceration because fresh solvent continuously replaces the saturated solvent within the column[29]. Solvent selection is important—nonpolar solvents like ether and hexane work well for oil extraction from seeds, while aqueous solvents are used for extracting polar constituents from leaves [30]. Percolation is a modified form of maceration in which the solvent slowly passes through a column of powdered plant material under gravity. The solvent is allowed to percolate for 24 hours, and the collected extract (percolate) is combined with expressed liquid from the marc to form the final extract [32]. A comparative study on extracting cannabidiol (CBD) from hemp biomass showed that percolation yielded higher concentrations than maceration, suggesting its potential for extracting lipophilic compounds from various botanical materials [33].

III. Decoction

Compared to maceration, decoction can improve the solubility of phytochemicals, such as alkaloids, flavonoids, and polysaccharides. However, decoction has limitations, including the presence of impurities in the crude extract and the inability to extract heat-sensitive or volatile components[29,30]. Decoction involves boiling plant material in water to extract heat-stable, water-soluble compounds. The process may last from minutes to hours and is followed by filtration. Decoction is suitable for woody materials such as roots, seeds, and bark[32]. A study by Ennaifer et al. optimized decoction conditions for *Pelargonium graveolens*, using response surface methodology to assess how time and temperature influence antioxidant activity and polysaccharide yield[34].

IV. Microwave-Assisted Extraction

Microwave-Assisted Extraction (MAE) utilizes non-ionizing radiation to extract plant metabolites. Microwave energy covers a frequency range of 300 MHz–300 GHz. Microwaves induce dipole rotation in organic molecules, causing the destruction of hydrogen bonds and enhances solvent penetration into the plant matrix, facilitating the extraction of plant metabolites[35]. Solvents with high dielectric constants are preferred for MAE because nonpolar solvents are transparent to microwaves and remain unheated[36]. In closed-vessel MAE, increasing the temperature from 60 to 120°C significantly enhanced the extraction efficiency. Since pressure is directly dependent on temperature, it allows for heating above the boiling point[30]. The optimal extraction time varies widely, ranging from 30 seconds to 20 minutes, although longer extraction time leads to degradation of phytochemicals. The microwave power was inversely proportional to irradiation time. Generally, longer exposure at lower power levels (e.g., 30-150 W) is considered more appropriate for most extraction cases, as it improves extraction efficiency while minimizing the risk of thermal degradation[37,38]. According to *Asma Khalf*, MAE of polyphenols from dried seed powder was determined using a FLEXIWAVE microwave oven. The optimized extraction conditions included a sample-to-solvent ratio of 1:20, extraction time, temperature, and ethanol concentration varied according to a Box-Behnken design, and a constant power of 1200 W[39]. Microwave-assisted extraction (MAE) of total phenolic contents (TPC) from E. indica using ethanol[40]. Microwave-assisted extraction (MAE) experiments on onion leaves were conducted using a PreeKem-M3 digestion system with a microwave frequency of 2450 MHz[41].

V. Hot Continuous counter current extraction method (Soxhlet extraction)

This technique involves circulating a fixed quantity of solvent through an extractor via evaporation and condensation. The extraction time for Soxhlet extraction is typically approximately 24 h at temperatures ranging from to 65-100°C. The choice of solvent is also crucial; inert and easy-to-remove solvents are preferred. However, samples with high water content cannot be extracted using this method, as it may cause degradation[30,32] [42]. The Soxhlet extraction apparatus consisted of a thimble-shaped filter paper containing the crude substance placed within a glass cylinder fitted with a siphon tube and inlet tube. A water condenser was attached to the cylinder, which was then inserted into the neck of the round-bottom flask containing the solvent. The solvent flask was heated in a water or sand bath, causing the solvent vapor to rise through the inlet tube and condense in the condenser. The condensed solvent then comes into contact with the crude substance and dissolves it. As the solution reached the top of the siphon tube, it was siphoned back into the flask, maintaining a continuous supply of solvent vapor and allowing the dissolved organic compounds to flow

back into the flask. Finally, the heating is stopped, and the solution is distilled to recover the solvent, leaving the extracted organic compound behind. Typical variables influencing Soxhlet extraction include the nature of plant material, particle size, solvent polarity, and extraction duration[43,44].

5. Evaluation of Antifungal Activity

For the development of effective Phytoconstituent-Loaded Transferosomes, it is crucial to assess the antifungal activity of the encapsulated compounds accurately. These methods provide the necessary data to evaluate the potency of the extracts and optimize transferosomes formulations.

I. Agar disk diffusion

The agar disk diffusion technique is widely employed for assessing the antimicrobial activity of plant extracts. Fungal colonies (8–24-hour cultures) grown on nutrient agar plates were selected and cultured in Mueller-Hinton Broth (MHB), Tryptone soy agar or Nutrient agar. The inoculum size typically consists of 10^7 CFU/mL for yeasts, equivalent to the McFarland 0.5 turbidity standard Filter paper disks impregnated with test compounds are placed on agar plates inoculated with fungal cells. The drying time for the impregnated disks was varied from 2 h to overnight under a laminar flow cabinet. Negative controls consisted of pure DMSO, sterile distilled water, or ethanol, while positive controls included antibiotics, such as vancomycin, amoxicillin, and amphotericin B discs. Following incubation at 48 hours at 25°C. The diffusion of chemicals from the discs into the agar medium inhibited the growth of microorganisms, resulting in distinct zones of inhibition around each disc. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of plant extract or pure phenolic acid that produced an inhibition zone around the disc[45,46].

II. Agar well diffusion

The agar well diffusion assay is analogous to the agar disk diffusion assay, which shares similar principles. Standardized bacterial and Candida broth cultures (0.5 McFarland standard) were streaked onto Mueller-Hinton agar (MHA) plates to create uniform lawn growth and allowed to dry at room temperature for 30 minutes[4748]. Mueller-Hinton Agar (MHA) plates were divided into quadrants and labeled. A sterile cork borer or tip is then used to create a cylindrical well (6-8 mm diameter) in the agar[49]. Extract at concentrations of 25%, 50%, 75%, and 100% were then added to the wells, allowing the solutions to diffuse into the MHA at room temperature for specified hour[50]. Pure solvents served as controls, whereas antibiotics were used as reference standards. The plates were incubated at 37°C overnight for yeast-like fungi or at room temperature for 48 hours for mold[51]. The antifungal activity of the extract was determined by measuring the diameters of the inhibition zones which were measured using a metal caliper and recorded in millimeters[52,5354]

III. Antimicrobial gradient method (E-Test).

The E-test is a combination of the diffusion and dilution methods. This technique involves creating a concentration gradient of the compound in the agar medium[55]. This method is specifically designed to determine the minimum inhibitory concentration (MIC)[56–58]. The test involves using a specialized test strip impregnated with gradient concentration of the antimicrobial agent(extract), which is then placed on an agar plate inoculated with the test organism[59]. After incubation, the antimicrobial activity is visible as an ellipse of inhibited growth around the strip, As the antimicrobial agent diffuses from the strip into the agar, a zone of inhibition forms and the MIC value is determined by the point where the ellipse intersects the scale[60,61]. Terbinafine minimum inhibitory concentrations (MICs) were determined using the gradient test method with Terbinafine Ezy MIC Strips. The strips showed an MIC range of 0.002-32 mg/mL. The terbinafine strips were placed on the agar, and MICs were determined after 5 days of incubation at 25°C[62]. This method offers flexibility and allows laboratories to test the drugs of their choice. However, with costs ranging from $2 to $3 per strip, this approach can become expensive when testing multiple drugs[63].

IV. Broth micro dilution

This approach involves serial dilutions of an antifungal agent in a liquid growth medium, which is then inoculated with a standardized fungal suspension. Stock solutions of plant extracts and the positive control drug prepared in dimethyl sulphoxide (DMSO)[64]. Two-fold serial dilutions of the plant extract were prepared in a specified concentration range and transferred to a 96-well microplate. Fresh fungal colonies were suspended in Mueller-Hinton broth to achieve a turbidity of 0.5 McFarland standard[65,66]. Inoculate each well containing the antibiotic solution and the growth control well with the fungal suspension[67,68]. After incubation, turbidity of the wells was checked visually or using an automated reader. The MIC can be determined by measuring fluorescence intensity or turbidity at specified wavelengths[63]. The concentration with a prominent decrease in turbidity was determined as the minimum inhibitory concentration (MIC)[69,70]. Recently, improved and validated colorimetric microdilution assays have been developed, using triphenyl tetrazolium chloride (TTC) as an indicator. These assays can correlate absorbance with the concentration of viable microorganisms and determine not only MIC but also 50% and 90% inhibitory concentrations (IC50 and IC90, respectively)[71,72]. Guidelines such as Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) provide a framework for testing the antimicrobial susceptibility of bacteria, yeast, and filamentous fungi[73,74].

V. TLC Bioautography

Bioautography can be effectively combined with thin-layer chromatography (TLC) to separate and identify bioactive compounds[75]. The test process involved the application of a specific amount of plant extract to a silica gel plate and developed using a suitable solvent system

to separate the phytochemicals. A suspension of the test bacteria was then sprayed or dipped onto a TLC plate. The bioautogram is incubated under suitable condition [76]. The bioautograms were developed by spraying the plates with p-iodonitrotetrazolium violet (INT) solution, resulting in white spots indicating inhibition zones against a pink-purple colored background [77]. These salts are converted into colored formazan by living microorganisms, indicating growth. The Rf values of the active spots with antifungal activity on the bioautograms were compared to identify the antifungal compounds[78]. Schmou et al. and sakunpak et al. developed TLC using silica gel G60 F254 and developed with mobile phase with multiple solvents[79,80].

VI. Time kill test

This test involves exposing microorganisms to varying concentrations of antimicrobial agents over a specific period, providing insights into time-dependent or concentration-dependent effects[75].Time-kill testing is the most effective method for evaluating the bactericidal or fungicidal effects of antimicrobial compounds and understanding their interactions with microbial cells[81]. The test involves incubating bacterial suspensions with test tubes or wells containing growth medium and specific concentrations of antimicrobial agents are prepared. The test microorganisms were then inoculated into each tube or well to ensure a consistent starting cell density. The tubes or wells are incubated under controlled conditions for predetermined time intervals, and samples are withdrawn at each interval (0-24 hours) and the percentage of dead cells is calculated relative to the growth control using the agar plate count method[82-84]. The colonies were counted and compared with the control in terms of cfu/mL[85]. A bactericidal effect is typically achieved with a 90% lethality rate at 6 hours or 99.9% lethality at 24 hours. Generally, samples were taken at 3, 6, 9, and 18 hours[86].

6. Transferosomes

Transferosomes are specially designed vesicular carrier systems that consist of an inner aqueous compartment enclosed by a lipid bilayer and an edge activator. This unique structure creates ultra-deformable vesicles with self-optimizing and self-regulating capabilities, allowing them to penetrate the skin through narrow pores to deform and pass through the

stratum corneum (SC) without significant depletion. Transferosomes up to 500 nm in size can spontaneously penetrate the SC[6,87]**.** The structure of a transferosome consists of an aqueous core surrounded by a complex lipid bilayer. These systems are formed by complexing phytoconstituents with phospholipids. The phospholipids used possess both hydrophilic and lipophilic properties, allowing them to interact with both water-soluble and fat-soluble constituents of the Phytoconstituent-Loadedand act as a drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents[88,89]. Transferosomes are composed of phosphatidylcholine, edge activator and other ingredients as shown in Table 3. The exact mechanism of transferosomes' enhanced delivery of active substances across the skin is not well understood but may include combination of Drug vectorization, Stratum corneum disruption or Penetration features of transferosome vesicles[90,91].

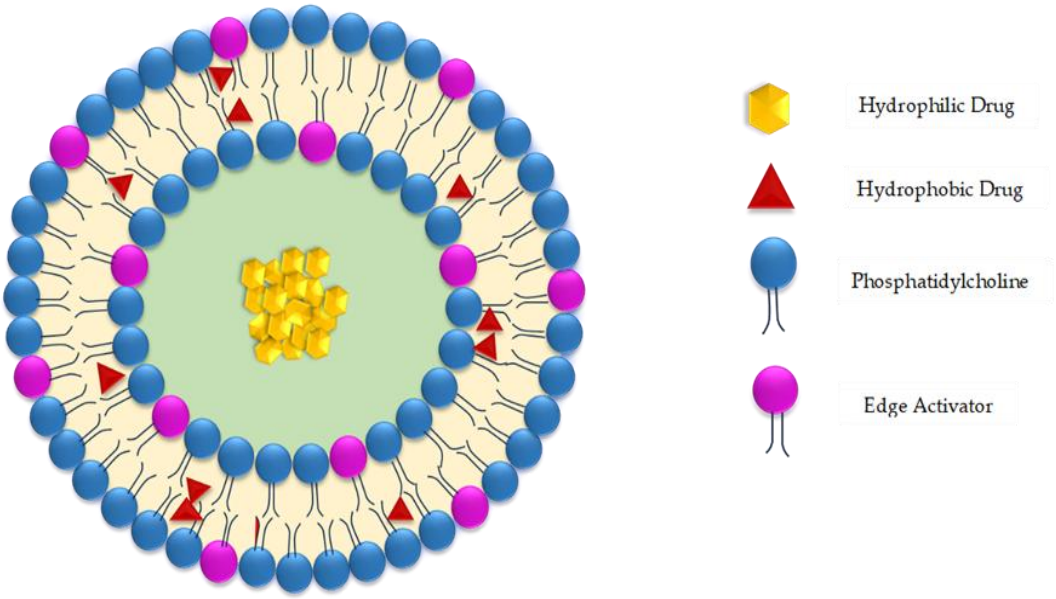

Figure 1: Transferosomes Structure

Polar lipids, such as those found in transferosomes, attract water due to their hydrophilic residues. As a result, lipid bilayers resist dehydration, and lipid vesicles move from dry locations to areas with high water concentrations. When a transferosomes suspension is applied to the skin surface, the lipid vesicles sense the osmotic gradient and move along it to avoid dehydration. To achieve this, transferosomes must be sufficiently deformable to pass through the narrow pores in the skin[88 92]. Transferosomes composed of surfactants have

optimal rheological and hydration properties, allowing them to overcome the skin penetration difficulty by squeezing themselves along the intracellular sealing lipids of the stratum corneum. Transferosomes demonstrate higher permeation efficiency compared to conventional liposomes, despite having a similar bilayer structure that facilitates the encapsulation of lipophilic, hydrophilic, and amphiphilic drugs[93]. They offer a promising solution for improving antifungal drug delivery systems are typically administered as creams or gels, offering non-invasive and patient-friendly treatments with improved compliance[94,95].

6.1 Composition of Transferosomes

Table 3: Key Components of Transferosomes Vesicles

Ingredients	Example	Role	Ref
Phospholipids	Soya Phosphatidylcholine Egg Phosphatidylcholine Phosphatidylserine Dipalmityl Phosphatidylcholine Distearyl Phosphatidylcholine	Provide lipid bilayer forming vesicle	88
Edge Activator (Surfactant)	Sodium Cholates Sodium Deoxycholate Tween80 Span60, Span 65	Provide flexibility, elasticity, Improve permeation as well as act as stabilizer	89
Buffering Agent	Saline Phosphate Buffer (Ph 7)	Hydrating the thin film, Adjust pH	91
Solvents	Ethanol Methanol Chloroform Water	Dissolve phospholipids and edge activators	96

6.2 Methods of Transferosomes Preparation: The selection of a suitable method is critical for achieving optimal transferosomes characteristics, such as size, stability, and encapsulation efficiency. Various methods are listed below.

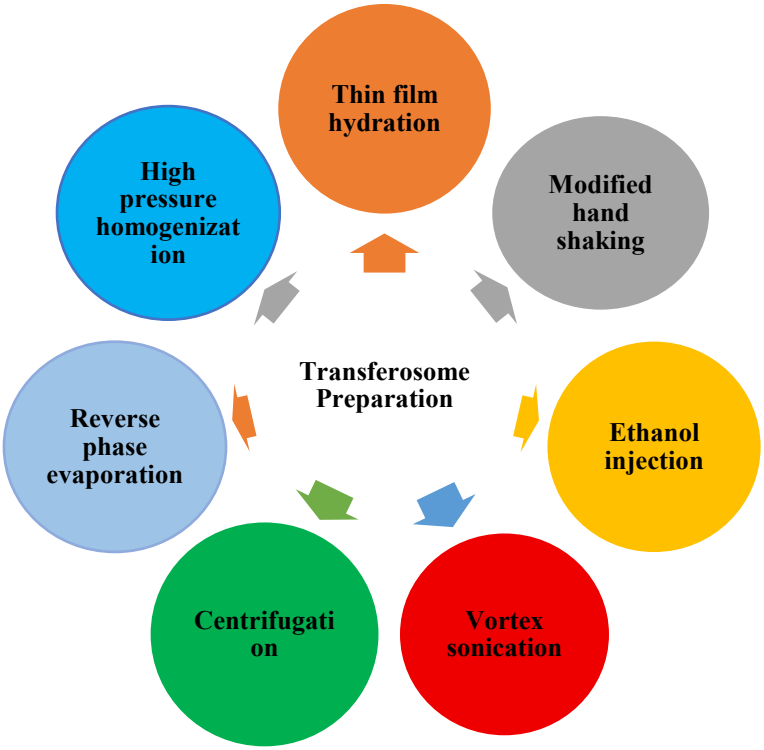

Figure 2: Transferosomes Preparation Methods

I. Thin film hydration method/ Rotary Evaporation sonication

The preparation of vesicles involves a multi-step process. Initially, phospholipids and edge activators are dissolved in a volatile organic solvent mixture, such as chloroform and methanol, in a round-bottom flask. Lipophilic drugs can be incorporated during this stage. Subsequent evaporation of the organic solvent under reduced pressure using a rotary vacuum evaporator yields a thin film. The deposited film is then hydrated with a buffer solution of appropriate pH, allowing for the incorporation of hydrophilic drugs. The resulting vesicles are then sonicated and homogenized through extrusion via polycarbonate membranes with decreasing pore sizes (200-100 nm) to obtain uniform, small vesicles[97,98].

II. Modified hand shaking method

The modified hand-shaking method, analogous to the rotary evaporation-sonication method, offers an alternative approach to preparing lipid-based formulations. Initially, a round-bottom flask is charged with an organic solvent, lipophilic drug, phospholipids, and edge activator, yielding a clear, transparent solution upon complete dissolution. Subsequently, the organic solvent is evaporated through manual agitation, while the flask is partially immersed in a thermostatically controlled water bath (40-60°C), resulting in the formation of a thin lipid film. Following overnight incubation to ensure complete solvent removal, the film is hydrated with an appropriate buffer solution at a temperature exceeding its phase transition temperature, facilitating the incorporation of hydrophilic drugs[96,99–103].

III. Vortex Sonication.

A novel approach to preparing transferosomes involves a straightforward mixing and sonication process. Initially, phospholipids, edge activator, and drug are combined in a phosphate buffer. The mixture is then vigorously agitated using vortexing, yielding a milky transferosome suspension. Subsequent sonication in a bath sonicator at room temperature, followed by extrusion through polycarbonate membranes of decreasing pore sizes (450-220 nm), results in a homogeneous transferosomal formulation[96,100,101,103–105].

IV. Ethanol injection

A novel method for preparing lipid-based formulations involves a solvent injection technique. Initially, a clear organic phase solution is obtained by dissolving phospholipid, edge activator, and lipophilic drug in ethanol via magnetic stirring. Concurrently, an aqueous phase is prepared by dissolving water-soluble substances in a phosphate buffer, allowing for the incorporation of hydrophilic drugs. Both solutions are heated to 45-50°C. The ethanolic phospholipid solution is then injected dropwise into the aqueous solution under continuous stirring. Finally, ethanol is removed via vacuum evaporation, and the resulting dispersion is sonicated to reduce particle size, yielding a uniform formulation[96,101–106].

V. High Pressure Homogenization Technique

A simplified method for preparing transferosomes involves a combination of ultrasonic shaking and high-pressure homogenization. Initially, phospholipids, edge activator, and drug are uniformly dispersed in a phosphate-buffered saline (PBS) solution or distilled water containing alcohol, followed by simultaneous ultrasonic shaking and stirring. The mixture is then subjected to intermittent ultrasonic shaking to facilitate further dispersion. Subsequent homogenization using a high-pressure homogenizer yields a uniform transferosomal formulation, which is then stored under suitable conditions[96,102].

VI. Reverse phase evaporation method

A novel method for preparing vesicular formulations involves a multi-step process. Initially, phospholipids and edge activator are dissolved in a mixture of organic solvents, such as diethyl ether and chloroform, in a round-bottom flask, allowing for the incorporation of lipophilic drugs. The solvent is then evaporated using rotary evaporation to yield lipid films, which are subsequently redissolved in an organic phase comprising isopropyl ether and/or diethyl ether. A two-phase system is formed by adding an aqueous phase, enabling the incorporation of hydrophilic drugs. Sonication using a bath sonicator transforms this system into a homogeneous water-in-oil (w/o) emulsion. Finally, slow evaporation of the organic solvent using rotary evaporation yields a viscous gel, which eventually forms a vesicular suspension[96,101–106].

VII. Centrifugation process

A lipid-based formulation can be prepared through a multi-step process. Initially, phospholipids, edge activator, and lipophilic drug are dissolved in an organic solvent. The solvent is then removed using rotary evaporation under reduced pressure, followed by vacuum drying to eliminate residual solvent. The resulting lipid film is subsequently hydrated with an appropriate buffer solution by centrifugation at room temperature, enabling the incorporation of hydrophilic drugs. The hydrated vesicles are then allowed to swell at room temperature, and the resulting multilamellar vesicles are further processed through sonication at room temperature to achieve the desired formulation[100,102,103].

6.3 Evaluation of Transferosomes

Various techniques provide crucial data on the physical and chemical properties of transferosomes, which are essential for predicting their performance in drug delivery.

A. Vesicle Size, Morphology and Zeta Potential

To determine vesicle size, measurements can be taken using Dynamic Light Scattering (DLS) technology. The vesicle solution is first mixed with a medium, and three readings are taken. Next, the sample is filtered using distilled water and a 0.2 mm filter. The filtered sample is then diluted with saline, and vesicle size is measured. Finally, specialised equipment such as the Malvern

Zetasizer and Transmission Electron Microscopy (TEM) is used to analyse vesicle size, distribution, structure and zeta potential. This value indicates the surface charge of transferosomes, influencing their interactions and stability[107–111].

B. Entrapment efficiency

To determine the percentage entrapment efficiency (%EE) of drugs in vesicles, two methods can be employed. The direct method involves ultracentrifugation to separate the vesicles from the supernatant, followed by disruption of the sedimented vesicles with a solvent. The resulting solution is then analyzed using HPLC or spectrophotometry to determine the drug content, and %EE is calculated accordingly.

$$\%EE = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug}} \times 100 \quad (1)$$

In contrast, the indirect method involves diluting and filtering the supernatant to measure the free drug concentration. %EE is then calculated by subtracting the free drug amount from the total added drug amount and dividing by the total added drug amount[108,110,112–114].

$$\%EE = \frac{\text{Amount of drug added} - \text{Amount of drug free}}{\text{Total amount of drug added}} \times 100 \quad (2)$$

C. Degree of Deformability

To evaluate deformability, a standardized method is employed, utilizing pure water as a reference. The transferosomal preparation is then sequentially passed through a series of microporous filters with precisely defined pore sizes, ranging from 50 to 400 nanometers. After each filtration step, the particle size and size distribution of the transferosomes are measured using the Dynamic Light Scattering (DLS) technique. By analyzing these measurements, the degree of deformability is quantitatively expressed, providing valuable insights into the formulation's potential to permeate through narrow pores and biological membrane [115,116]. The degree of deformability is expressed as :

$$D = J \times \frac{rv}{rp} \quad (3)$$

Where,

D= Degree of deformability,

J = Amount of suspension extruded during 5 min,

rv = Size of the vesicle

rp = Pore size of the barrier.

D. In-vitro skin permeation studies

Franz diffusion cells are used to study skin permeation. Animal skin, such as rat skin or porcine skin, is used to study skin penetration. The skin is mounted on a donor chamber, with the dorsal surface facing the chamber, allowing researchers to assess how the formulation penetrates the skin. The selected membranes, mimicking the stratum corneum, are mounted on the receptor compartments. The receptor compartments contain phosphate buffer saline solution, stirred by a magnetic bar, and maintained at 37 ±0.5°C to simulate blood circulation. Transferosomal formulation is added to the donor compartment, and the cell is left open to mimic non-occluded conditions. At regular intervals, samples are withdrawn from the receptor medium, replaced with fresh medium to maintain sink conditions, and analyzed using HPLC or spectroscopic methods[114,115,117].

7. Conclusion

The advent of transferosomes technology represents a promising strategy for enhancing the delivery of phytoconstituents in the treatment of topical fungal infections. The ability of these ultra-deformable vesicles to penetrate the stratum corneum significantly improves drug bioavailability at infection sites, which is crucial in the context of rising antifungal resistance. Furthermore, the integration of potent herbal remedies with transferosomes carriers may lead to synergistic effects, amplifying the therapeutic efficacy while potentially reducing side effects associated with conventional antifungal therapies. Methods like thin film hydration and ethanol injection are commonly used to prepare herbal transferosomes. Characterization techniques such as vesicle size, entrapment efficiency, and in vitro release studies are important for optimizing transferosomes formulations. However, challenges remain in optimizing formulation parameters, scaling production, and ensuring therapeutic consistency. Future research should focus on clinical applications, long-term efficacy studies, and the development of protocols for standardized applications. Ultimately, herbal transferosomes have the potential to revolutionize the landscape of topical antifungal treatment, providing a safe and effective alternative for managing superficial fungal diseases.

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