RESEARCH PAPER

Formulation Development of Herbal Liposomal Gel Containing Plant Extracts for Antifungal Effect

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Abstract
The present study aimed to develop and evaluate a herbal liposomal gel containing selected plant extracts for enhanced antifungal activity. Fungal infections of the skin are among the most common dermatological disorders, and the increasing resistance associated with conventional antifungal agents has created a need for safer and more effective herbal alternatives. Herbal medicines possess significant therapeutic potential due to the presence of bioactive phytoconstituents with antifungal, anti-inflammatory, and antioxidant properties. However, poor solubility and limited skin penetration of herbal extracts often reduce their therapeutic efficacy. To overcome these limitations, liposomal drug delivery systems were employed in the present investigation. Selected medicinal plant extracts possessing reported antifungal activity were prepared using suitable extraction methods and subjected to preliminary phytochemical screening. Liposomes were formulated using phospholipids and cholesterol by thin-film hydration technique and subsequently incorporated into a carbopol-based gel system. The prepared herbal liposomal gel was evaluated for various physicochemical parameters including appearance, pH, viscosity, spreadability, homogeneity, drug content, entrapment efficiency, and in-vitro diffusion studies. Antifungal activity of the optimized formulation was assessed against standard fungal strains using cup plate or agar well diffusion method. The formulated liposomal gel showed satisfactory physicochemical characteristics with good stability, uniformity, and enhanced diffusion profile. The optimized formulation demonstrated significant antifungal activity compared to the conventional herbal gel, indicating improved penetration and sustained release of active phytoconstituents through the liposomal carrier system. The study concluded that herbal liposomal gel can serve as an effective and promising topical delivery system for the treatment of fungal infections with improved therapeutic efficacy and reduced side effects.

Keywords: Herbal liposomal gel, plant extracts, antifungal activity, liposomes, topical delivery, phytoconstituents, fungal infections.

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Introduction

Topical drug delivery is the technique of applying drugs directly onto the skin or mucosal surfaces to provide a localized therapeutic impact. Commonly used for the treatment of dermatological diseases, eye infections, nasal and vaginal issues, and for pain relief [1]. Among various clinical applications, topical drug delivery systems are among the most frequently applied because skin is one of the most accessible pathways for drug delivery. On the other hand, topical medical treatments range from simple liquids and ointments to multiphase nanotechnology-based therapies. Advantage of topical administration system is bypass first pass metabolism. Topical preparations have other advantages as well: they allow one to bypass the risks and inconveniences of IV therapy and the variableabsorption conditions such as alterations in pH, if enzymes are present or not, stomach emptying time [2]. Pharmaceutical dosage form, from semisolids to liquid preparation, sprays, and solid powders, are used as topically acting medications. Semisolid preparations for topical administration of drugs consist mainly of gels, creams, anointments [3]. A gel-based product called topical gel is administered straight to the skin or mucous membranes. Designed to provide active components to a specific location for localized therapy, it provides advantages including pain alleviation, inflammation reduction, or treatment of skin disorders [4]. A gel is a two component, crosslinked three-dimensional network of structural elements separated by a suitable, but comparatively massive content of fluid to make a boundless inflexible network structure that clogs the fluid constant. stage inside. A gel is a state of matter intermediate between those of a solid and a liquid that contains properties of both solids and liquids, known as viscoelasticity The structural components forming a gel network may be inorganic particles or organic macromolecules, generally-sized polymers.(5) The topical delivery system is occasionally used if other routes of drug administration fails or primarily it is used in pain relief, contraception and urine incontinence. In last decades the approach towards treating any sort of disease has been administration of anti-biotic drugs in

human body by various routes such as oral, sublingual, rectal, parental, topical, aerosolic etc. [6] Topical drug delivery is defined as the topical application of the drug containing formulation to the skin for the treatment of cutaneous disorders (eg, acne) or for the treatment of the cutaneous lesions of a systemic disease (eg, psoriasis), with the aim of restricting the pharmacological or other action of the drug to the skin surface or to the skin [7]. The various medicinal plants showed beneficial physiological activity on human beings. Opium, aspirin, digitalis, and quinine are just a few of the phytopharmaceuticals with a long history of treatment usage. These organic substances served as the foundation for the discovery of modern medications. Echinocandines and sordarines, two antifungal natural origin compounds, have recently entered the clinical study [8]. A novel drug delivery system uses a ground-breaking strategy to address the drawbacks of traditional drug delivery systems. Only a few unique herbal compositions have been examined using proactive and plant-based delivery techniques, such as liposomes, nanocapsules, ethosomes, and microspheres [9]. The main benefits of herbal nanocarriers over traditional formulations include improved solubility, increased bioavailability, toxicity prevention, higher pharmacological action, The Indian medical system is firmly rooted in the skill of using herbs due to their therapeutic benefits. Pharmaceutical companies frequently use herbal medicine as a complementary form of therapy. However, the traditional drug delivery technique of these herbal medicines limits the efficacy of the bioactive from the herbal source. Several potent antifungal medications exist, but their therapeutic benefits are constrained by their high toxicity or undesirable physicochemical features [10]. Liposomes are spherical vesicles having an aqueous core enclosed by one or more phospholipid bilayers or lamellae. Liposomes are most frequently classified on the basis of their size (small, large and giant vesicles), number of bilayers (uni-, oligoand multi-lamellar) and phospholipid charge (neutral, anionic or cationic). The objective of proposed work is to prepare herbal liposomal gel for antifungal activity. The goal of the current study is to create a herbal liposomal gel that is safe, efficacious, and stable for topical antifungal treatment [11]. To create a better herbal delivery system, the research combines pharmaceutical formulation, nanotechnology, phytochemistry, and microbiological assessment. The activities of liposomes as topical medication delivery systems to the skin vary based on their size, lipid and cholesterol composition, ingredient percentage, lamellarity, and surface charge. liposomes can overcome several barriers to cutaneous medication delivery, improve penetration through the stratum corneum, and reduce systemic effects through their localizing properties.

Material and methods

The plants *Tinospora cordifolia (Giloy), Guduchi, Oscimum tenuiflorium (Tulsi), Allium Sativum (Garlic)* were collected from market or nursary. In view of its diverse medicinal applications and in order to ensure the quality, authenticity and assay, and in view of lack of pharmacognostic study the present investigation was undertaken with an objective to evaluate *Tinospora cordifolia (Giloy), Guduchi, Oscimum tenuiflorium (Tulsi), Allium Sativum (Garlic)* on various pharmacognostic parameters, such as macroscopic, physiochemical, and phytochemical studies of the plant [12-13].

Liposomal Drug Delivery System

By using thin film hydration method were prepared multi lamellar liposome containing Tinospora cordifolia (Giloy) (50mg), Oscimum tenuiflorium (Tulsi) (50mg) and Allium Sativum (Garlic) (50mg) extract. The plant etract (150 mg), soya lecithin (100 mg) and cholesterol (15 mg) were dissolved in chloroform and methanol mixture in ratio (9:1). These above solutions pour in round bottom flask of rotary flash evaporator. In rotary flash evaporator the organic solvent evaporates at 60ºc, for 15min. at 90rpm. After evaporating organic solvent thin layer which are form on inner surface of round bottom flask. These thin layers dried overnight by using vacuumed oven. Then this thin lipid layer suspension in phosphate buffer saline (PBS) having PH 7.4 by vertexing for 10min.and then it was allowed to hydrate for 1hr at 70ºc, 90 rpm. Then this liposomal suspension centrifuge by using ultra-centrifugation Machin at 3000 rpm for 30 min. Then the settle liposome again centrifuge in PBS. Then this suspension of liposome sonicates for 15 min at 65ºc to get small unilamellar vesicle (SUV) [14].

Carbopol is a water-soluble polymer which acts as powerful, gelling thickener useful for making clear gels. In order to achieve desired gel consistency and spreadability, different concentrations of Carbopol 940 [15].

Formulation of herbal antifungal liposomal gel

Herbal antifungal liposomal gel was prepared by incorporating methanolic extract of plants liquid in to optimized Carbopol 940 gel. On the other hand, accurate amount of methanolic extracts of all plant extract (2%), propylene glycol- 400, methylparaben and propylparaben were added to the Carbopol 940 dispersion. Triethanolamine was added dropwise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. It was then stirred by using propeller for 2 hours at 500rpm. After stirring, the prepared gel appeared to

be homogenous and devoid of air bubbles. The prepared gel was kept at room temperature for 24 hours [16-17].

Table 1: Formulation of herbal gel

Ingredients	L4GB1H1	L4GB1H2	L1GB1H3
Optimized liposomes containing plant extract (L4)	200 mg	250 mg	300 mg
Carbopol 940	200 mg	200 mg	200 mg
Propylene glycol	5 ml	5 ml	5 ml
Methyl Paraben	150 mg	150 mg	150 mg
Propyl paraben	300 mg	300 mg	300 mg
Triethanolamine	5 ml	5 ml	5 ml
Water	q. s	q. s	q. s

Evaluation of herbal antifungal liposomal gel

The prepared herbal antifungal liposomal gel was subjected to physical characterization such as color, appearance, pH, viscosity, spreadability. It was also evaluated for its stability property, antimicrobial activity and in vivo skin irritation study [18].

Physical appearance

The formulated herbal antifungal liposomal gel was inspected visually for their color, odor, homogeneity and consistency. All developed gels were tested for homogeneity by visual inspection after gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Measurement of pH

The pH of various formulations was determined by using digital pH meter. One gram of gel was dissolved in 100ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate.

Determination of viscosity

The measurements of viscosity of prepared herbal antifungal liposomal gel were carried out with Brookfield viscometer (Brookfield viscometer RVT) with spindle No.62 [19].

Spreadability

Spreadability denotes the extent of area to which the herbal antifungal liposomal gel readily spreads on application to skin or the affected part. Two sets of glass slides of standard dimensions were taken. The gel formulation was placed over one of the slides. The other slides were placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 6.0 cm along the slide.100gm weight was placed upon the upper slides so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and in such a way that only the upper slide slip off freely by the force of weight tied to it. A 20gm weight was tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. The experiment was repeated three times and the mean time taken for calculation [20].

Spreadability was calculated by using the following the formula:

$$S = \frac{(M \times L)}{T}$$

Where, S = Spreadability, M = Weight in the pan (tied to the upper slide) L = Length of the glass slide, T= Time (in sec) taken to separate the slides.

Bloom Strength

The bloom strength of the herbal antifungal liposomal gel was determined by means of Texture Analyzer equipped with 5 kg load cell using a cylindrical probe of 0.5 diameter as fixture. The sample in the container was placed centrally on the platform beneath the cylindrical probe. After calibrating the height of the probe, the test was commenced. A trigger force of 10 g was used for the study [21].

Extrudability

The herbal gels were incubated at room temperature for 2 h before measuring their extrudability using an HDP/FE forward extrusion cell of the TA-XT2 Texture Analyzer equipped with a 5 kg load cell. Prior to measurement, the gel was manually stirred and loaded (100 g) into the cell. The compression force was measured at the following conditions: pre-test speed 1 mm/s, test speed 1 mm/s, trigger force 10 g, post-test speed 10 mm/s, compression distance 20 mm, and outlet diameter of extrusion cell 3 mm [22].

Antifungal activity of the optimized herbal gel

The following Standard cultures of American Type Culture Collection (ATCC) strains were used in the study:

Standard fungal cultures were obtained from ATCC and maintained on Sabouraud Dextrose Agar (SDA) slants. Commonly used strains include:

1. Candida albicans (ATCC 10231)
2. Aspergillus niger (ATCC 16404)
3. Aspergillus fumigatus (ATCC 204305)

Antifungal activity by cup plate method

The sterile Petri dishes were filled with Muller Hinton Agar medium which was then inoculated with a suitable dilution of a test organism Candida

albicans (ATCC 10231), Aspergillus niger (ATCC 16404) and Aspergillus fumigatus (ATCC 204305). Four cylinder or cups were made in the medium with the sterile borer in each plate. The formulated polyherbal gel, standard disc and solvent control were prepared. A uniform amount of 0.2 ml solution was added to the cup and incubated at 37ºC for 24 hrs. The well diffusion test was performed in triplicates and antimicrobial activity was expressed as the mean of inhibition in diameter(mm) [23].

Result and discussion

The liposomal formulations containing different combinations of Tinospora cordifolia (Giloy), Ocimum tenuiflorium (Tulsi), and Allium sativum (Garlic) were prepared using soya lecithin and cholesterol as lipid components. Each formulation contained a fixed lipid ratio (Soya lecithin 100 mg and cholesterol 15 mg), while the herbal drug combinations were varied to evaluate the effect of composition on Herbal antifungal liposomal gel containing *Tinospora cordifolia (Giloy), Guduchi, Oscimum tenuiflorium (Tulsi), Allium Sativum (Garlic)* extracts was incorporated into optimized 200 mg Carbopol 940 gel base with other ingredients. Different concentrations of optimized liposomes containing plant extract (L4) such as 200 mg, 250 mg and 300 mg were incorporated in to Carbopol gel base.

Table 3: Physical appearance of formulated gel

Parameters	L4GB1H1	L4GB1H2	L1GB1H3
Physical appearance	Transparent yellow gel	Transparent yellow gel	Transparent yellow gel
Color	Pale yellow	Pale yellow	Pale yellow
Homogeneity	Absence of aggregates	Absence of aggregates	Slight aggregates
pH	5.9	5.7	5.8
Viscosity [cps]	1428±0.1	1425±0.75	1358±0.25
Spreadability (gm.cm/sec)	19.37	21.35	22.13

The use of soya lecithin and cholesterol as lipid components allowed for the effective preparation of seven liposomal formulations. These formulations contained various combinations of Tinospora cordifolia (Giloy), Ocimum tenuiflorium (Tulsi), and Allium sativum (Garlic). A constant lipid composition of soya lecithin (100 mg) and cholesterol (15 mg) was included in each formulation. However, the content of herbal extracts was altered in order to investigate the impact that these extracts had on the physicochemical attributes and antifungal efficacy of the liposomes. For the purpose of preparing herbal antifungal liposomal gels, the improved liposomal formulation (L4) was further integrated into the Carbopol 940 gel base. There were three different concentrations of optimized liposomes that were put into the gel basis. These concentrations were 200 mg, 250 mg, and 300 mg, and they were each given the code L4GB1H1, L4GB1H2, and L1GB1H3 accordingly. Physical appearance, color, homogeneity, pH, viscosity, and spreadability were taken into consideration when evaluating the gels that were created. Within the gel matrix, all of the formulations displayed a translucent yellow appearance with a pale-yellow tint, which indicated that the liposomes were distributed evenly across the matrices. The formulations L4GB1H1 and L4GB1H2 exhibited a high degree of homogeneity and did not include any aggregates. On the other hand, formulation L1GB1H3 displayed a modest degree of aggregation, which indicated a relatively lower level of stability. All of the gel formulations had a pH that ranged from 5.7 to 5.9, which is appropriate for topical administration and is compatible with the pH of the skin. The viscosity readings were discovered to fall within the range of 1358±0.25 to 1428±0.1 cps, which signifies that the consistency is satisfactory and the application process is straightforward. In comparison to the other formulations, L4GB1H1 had the highest viscosity, measuring at 1428±0.1 cps. On the other hand, L1GB1H3 displayed a viscosity that was somewhat lower. 19.37 to 22.13 grams per square centimeter per second was the spreadability of the formulations. Although formulation L1GB1H3 shown the highest spreadability (22.13 gm.cm/sec), formulation L4GB1H2 demonstrated the second highest spreadability (21.35 gm.cm/sec), and formulation L4GB1H1 demonstrated the lowest spreadability (19.37 gm.cm/sec). Based on the findings, it appears that an increase in the concentration of liposomes had an effect on the rheological behavior and spreadability features of the gel formulations. Overall, formulation L4GB1H2 had satisfactory physicochemical features, including good homogeneity, ideal pH, optimal viscosity, and acceptable spreadability. These qualities indicate that the formulation has the potential to be an efficient herbal antifungal liposomal gel for topical application. The effectiveness of the antimicrobial agent was evaluated by measuring and contrasting the diameter of the zones of inhibition, which were measured in millimeters. The zone of inhibition is the clear region that surrounds the well and includes an antimicrobial agent. This defined zone can be found surrounding the well. It is well knowledge that the antimicrobial agent's effectiveness increases in

proportion to the size of the zone of inhibition. The herbal antifungal liposomal gel (L4GB1H2) that was produced was evaluated for its antimicrobial properties against several organisms, including Staphylococcus aureus, Escherchia coli, and Candida albicans. Additionally, it was compared with traditional treatments, such as Gentamicin (10µg) and Fluconazole (25 µg). Based on the findings, it was determined that the zone of inhibition was satisfactory, however it was lower than the standard.

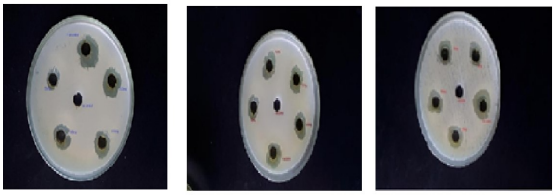

Figure 2: Measurement of Zone of inhibition of formulated herbal antifungal liposomal gel

Conclusion

Through the use of soya lecithin and cholesterol as lipid components, the current research was able to successfully synthesize and assess a herbal antifungal liposomal gel that contained extracts of Tinospora cordifolia, Ocimum tenuiflorium, and Allium sativum. An investigation into the influence of herbal extracts on physicochemical qualities and antibacterial activity was carried out by producing seven liposomal formulations using a variety of different combinations of herbal extracts. Formulation L4 was chosen as the optimal formulation among them, and it was subsequently mixed into a Carbopol 940 gel basis at a variety of concentrations. For topical application, the gels that were formulated exhibited physicochemical features that were adequate. These characteristics included a translucent appearance, a pale-yellow color, good homogeneity, an appropriate pH, an acceptable viscosity, and a spreadability that seemed desired. Therefore, the pH values were found to be within the skin-compatible range, which indicates that they are safe for usage on the dermis. Optimal qualities were demonstrated by formulation L4GB1H2, which also exhibited improved stability and was simpler to apply. An considerable antibacterial activity was established by the improved formulation against Staphylococcus aureus, Escherichia coli, and Candida albicans, with zones of inhibition that were satisfactory. Due to the synergistic action of herbal extracts and the improved penetration afforded by the liposomal delivery technology, the formulation demonstrated significant inhibitory capability, despite the fact that its activity was lower than that of common medications such as fluconazole and gentamicin.

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