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DEVELOPMENT, CHARACTERIZATION AND *IN-VITRO* ANTIFUNGAL EVALUATION OF PLANTEROSOMAL GEL OF *GANODERMA LUCIDUM*

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Keywords:

Planterosomal gel, *Ex-vivo* permeation study, *In-vitro* antifungal activity, *Ganoderma lucidum*

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ABSTRACT: Planterosomal gel exhibit better pharmacokinetic and pharmacodynamic profile than the conventional herbal extracts. The objective of the present research work was to develop, characterize and evaluate the *in-vitro* antifungal activity of planterosomal gel in comparison to pure plant extract. Planterosomal gel of *Ganoderma lucidum* was prepared by incorporating gelling agent in planterosomal suspension. Planterosomal gel of *G. lucidum* was subjected to different evaluation parameters like pH, spreadability, *in-vitro* release study, *ex-vivo* permeation study, and *in-vitro* antifungal activity. Results of *in-vitro* release studies and *ex-vivo* skin permeation studies showed that the planterosomal gel releases the active ingredients in a controlled manner and showed higher skin permeability in comparison to *G. lucidum* gel. The optimized planterosomal gel showed moderate antifungal activity when compared with a standard antifungal agent. So, it was concluded that the planterosomal gel is an advanced form of herbal formulation which enhances the retention time and skin permeability when applied topically.

INTRODUCTION: The formulation and delivery systems for topical dosage form is a challenging task for the formulation scientist and difficult to understand the protective mechanisms of skin such as the stratum corneum present in the skin which is highly lipophilic in nature and does not allow the entry of hydrophilic substances through this layer[1]. The semisolid preparations are mostly administered for dermatological actions. Topical gels are usually formulated by polymers that improve the appearance of the formulation and easily washed from the skin by water.

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Many of the synthetic drugs, used for treating skin disorder may sometimes cause an allergic reaction to the skin, so nowadays many of the herbal extracts are used which have greater therapeutic benefits and less adverse effects than the synthetic drugs [2]. For the improvement of patient acceptability, skin penetrability, bioavailability and to provide controlled release of active ingredients, a study on planterosomal gel will be advantageous. The topical route is a promising route for the delivery of drugs for local and systemic effect. The delivery of drugs onto the skin surface is considered as an effective therapy for local dermatological diseases.

Drugs delivered by topical route penetrate deeper into the skin and therefore give better absorption. The main benefit of this delivery system is to bypass first-pass metabolism [3]. Fungal diseases are commonly called as mycoses.

Most of the incidence of fungal infections increase due to the weak immune system related to HIV, cancer and other diseases. Fungi that cause skin diseases are called dermatophytes. Dermatophyte does not refer to a particular group of fungi but they attack the dermis or skin. Fungal diseases are mostly encountered in day to day life, which is prevented through the conventional dosage form like antifungal gels and creams *etc*. Since, delivery of the drug to the internal part of the skin still remains troublesome due to the protective structure of the skin [4, 5, 6]. This work aims to develop, characterize and *in-vitro* antifungal evaluation of planterosomal gel of *Ganoderma lucidum*.

MATERIALS AND METHODS:

Materials: *Ganoderma lucidum* extract was purchased from Navchetna health care Delhi. Carbopol 940 and Hydroxypropyl methylcellulose was obtained as a gift sample from Ottochemika. Propylparaben was obtained as a gift sample from Titan Biotech. All other chemicals and solvents were of analytical grade.

Methods:

Development of Planterosomal Gel of *G. lucidum*: Planterosome of *G. lucidum* was prepared by reacting the herbal extracts with different ratios of soya lecithin and cholesterol by using thin-film hydration method. Different batches of gels were prepared by the optimized formulation of *G. lucidum* planterosome. For the preparation of gel, different ratios of Carbopol 940 and HPMC were dispersed in planterosomal suspension. This solution was continuously stirred at 500 rpm for 30 min to get the planterosomal gel. Propylparaben (0.1%) was used as a preservative.

Characterization of Planterosomal Gel: [7-11]

Physical Evaluation of Gel:

pH: The pH of the prepared formulations after addition of all the ingredients was measured by using calibrated pH meter.

Rheological Studies: Viscosity of different gel formulations were determined by Brookfield Viscometer (De-V viscometer) in spindle no 62. At particular rpm the maximum torque and its respected reading in centipoises was noted down and the average of reading was used to calculate the viscosity.

Spreadability Test: 0.5 gm gel of each formulation was taken and pressed between two glass slides and kept aside for 5 min and diameters of each spread circles were measured in cm. The results obtained are an average of three determinations.

Homogeneity: The gel formulations were tested for the homogeneity by visual appearance and by touch.

Drug Content Analysis: 1.0 gm of the gel was dissolved in 100 ml phosphate buffer pH (5.5) and shaken vigorously for 2 h in mechanical stirrer for complete solubility of the drug. After that, the solution was filtered and 1 ml of sample was withdrawn and diluted to 10 ml in a volumetric flask and the absorbance was taken in UV spectrophotometer at 281 nm by using phosphate buffer (pH 5.5) as blank.

***In-vitro* Release Studies:** *In-vitro* drug release from the gel formulation was studied by the diffusion cell. The diffusion medium was 100 ml of phosphate buffer pH (5.5), stirred at 50 rpm at 37 ºC ± 0.5 ºC. One portion of the diffusion tube was wrapped by a dialysis membrane. 1 gm of gel formulations were filled in test tubes covered with a dialysis membrane and was placed in such a position that it touches the diffusion medium present in the receptor compartment. Samples were withdrawn at predetermined intervals for 10 h from diffusion medium and analyzed by UV spectrophotometer at 281 nm using phosphate buffer pH (5.5) as blank.

***Ex-vivo* Skin Permeation Studies:**

Preparation: Ethical clearance for performing experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC) (1452/PO/Re/S/11/CPCSEA/I/05) prior to the initiation of the experiment and the care of the laboratory animals was taken as per the CPCSEA regulations. The rat abdominal skin was surgically removed, cut into 2 cm^2 area and adhered fat was carefully removed. Hairs remaining on the skin were trimmed away. After that epidermis was withdrawn from the dermis by soaking the skin in 2 M sodium bromide solution for 6-8 h. The epidermis was then washed, wrapped and stored for further use.

***Ex-vivo* Skin Permeation Studies:** The *ex-vivo* permeation studies were performed by the use of Franz diffusion cell. The receptor compartment was filled with phosphate buffer pH 5.5 (diffusion medium) and receptor phase stirred at 50 rpm, maintained at 37 ± 0.5 ºC. 1 gm of the gel was placed in the donor compartment and covered with aluminum foil to prevent drying out.

Samples were withdrawn at a predetermined interval for up to 8 h, filtered and analyzed by UV-VIS spectrophotometer at 281 nm by taking phosphate buffer pH (5.5) as a blank. The receptor phase volume was replaced with the same volume of fresh receptor fluid to maintain sink conditions.

***In-vitro* Antifungal Activity of Planterosomal Gel:** [12, 13, 14] *In-vitro* antifungal activity of planterosomal gel and *G. lucidum* were performed by using agar well diffusion method.

Preparation of Culture Medium: Sabouraud's dextrose agar media (Hi-Media) was used for *in-vitro* antifungal activity. For the preparation of media dextrose, peptone and agar were accurately weighed, dissolved in distilled water and autoclaved at 121 ºC for 15 min. pH of the media was maintained at 5.6.

Standard Preparation: Fluconazole was used as a standard antifungal agent and prepared in sterile distilled water to give a final concentration of 1 mg/ml.

Sample Preparation: Planterosomal gel and *G. lucidum* were dissolved in sterile distilled water to give the final concentration of 20 mg/ml.

Preparation of Inoculum: The suspension of fungus was prepared by Mac-Farland Nephelometer Standard method. For the preparation of fungal suspension culture of *C. albicans* was used. The inoculum was prepared by suspending the isolated colony in 2 ml of 0.85% w/v of normal saline solution. It was then mixed slowly to form a smooth suspension.

Procedure for *in-vitro* Antifungal Activity: The prepared Sabouraud's dextrose agar media (Hi-Media) was poured in 5 sterile Petri plates and allowed to solidify. After that, the prepared inoculum was poured onto the surface of agar plates and spread by a glass spreader. By the use of flamed sterile borer (21 mm in diameter), the medium was bored and 0.1 ml of each standard and test samples were added in each bore. A control having only sterile distilled water was also maintained. The above procedure was carried out in aseptic condition in Laminar-air flow. The plates were then incubated at 28 ºC for 72 h. Finally, the zone of inhibition in each plate was recorded and the values of the test were compared with the standard sample.

RESULT AND DISCUSSION:

Development of Planterosomal Gel of *G. lucidum*: Four different formulations of *G. lucidum* planterosomal gel were formulated by using different ratios of the gelling agent with planterosomal suspension as per **Table 1**.

TABLE 1: COMPOSITION OF PLANTEROSOMAL GEL OF *GANODERMA LUCIDUM*

Ingredients	F1	F2	F3	F4
Planterosomal suspension (ml)	10	10	10	10
Carbopol 940 (% w/v)	0.2	0.3	0.4	0.5
HPMC (% w/v)	0.5	0.5	0.5	0.5
Propylparaben (% w/v)	0.1	0.1	0.1	0.1

Characterization of Planterosomal Gel:

Physical Evaluation of Gel: Physical evaluation of *G. lucidum* planterosomal gel such as pH, spreadability, viscosity, and homogeneity was carried out, and results are shown in **Table 2**.

TABLE 2: PHYSICAL EVALUATION OF PLANTEROSOMAL GEL

Formulation	Physical Evaluation			
	pH	Viscosity (cp)	Homogeneity	Spreadibility (cm)
F1	5.49 ± 0.01	26155	Homogeneous	4.53 ± 0.057
F2	5.38 ± 0.005	22142	Homogeneous	4.23 ± 0.057
F3	5.51 ± 0.012	28532	Homogeneous	4.06 ± 0.11
F4	5.54 ± 0.011	27165	Homogeneous	3.98 ± 0.01

Drug Content Analysis: % drug content of all planterosomal gel formulations shown in **Table 3** and **Fig. 1**. Among all the formulations highest drug content was observed with formulation F4 (0.5%).

TABLE 3: DRUG CONTENT ANALYSIS OF PLANTEROSOMAL GEL

Formulation	F1	F2	F3	F4
% Drug content	80.48	83.34	88.54	90.71

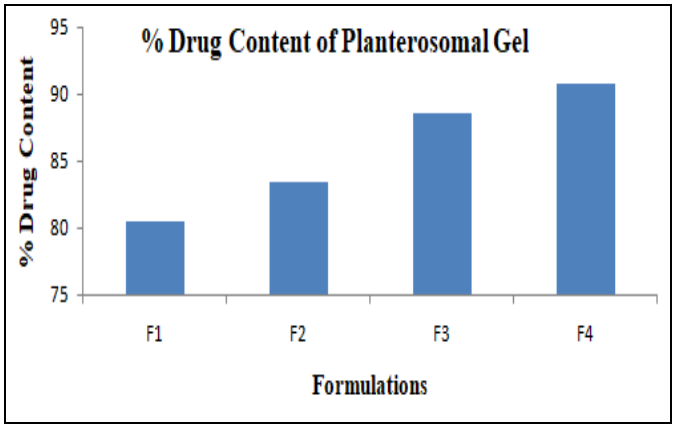


FIG. 1: % DRUG CONTENT OF *G. LUCIDUM* PLANTEROSOMAL GEL

FIG. 2: *IN-VITRO* RELEASE STUDIES OF *G. LUCIDUM* PLANTEROSOMAL GEL

***In-vitro* Release Studies:** *In-vitro* drug release from the gel formulation was studied by the diffusion cell as shown in **Fig. 2.** *In-vitro* release profile of planterosomal gel was showed in **Fig.3**.

***Ex-vivo* Skin Permeation Studies:** The *ex-vivo* skin permeability of Planterosomal gel was comparatively evaluated with *G. lucidum* gel. The *ex-vivo* study was performed using the abdominal skin of albino Wistar rat placed in the Franz diffusion cell.

It was observed that the planterosomal gel shows higher skin permeability across the rat abdominal skin when compared to the *G. lucidum* gel as shown in **Fig. 4**.

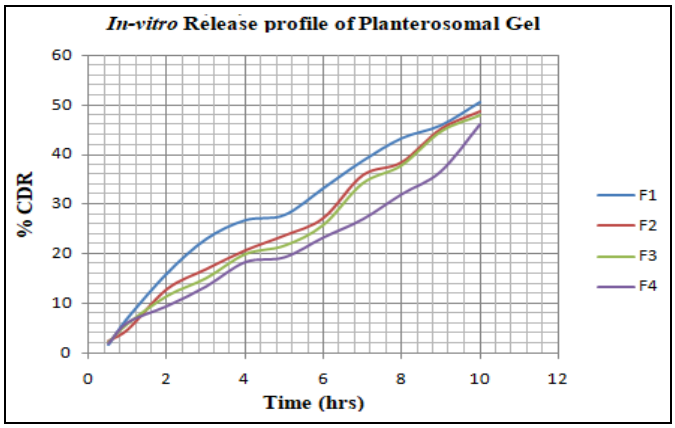


FIG. 3: *IN-VITRO* RELEASE PROFILE OF PLANTEROSOMAL GEL

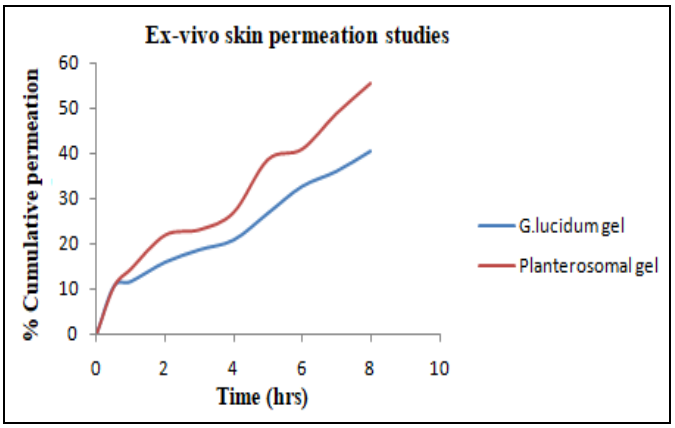
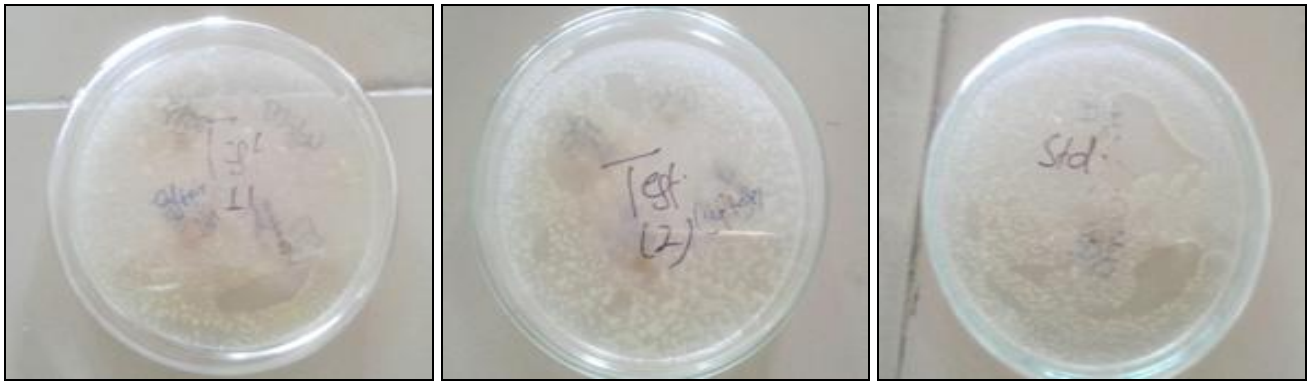


FIG. 4: *EX-VIVO* SKIN PERMEATION STUDIES OF *G. LUCIDUM* AND PLANTEROSOMAL GEL

***In-vitro* Antifungal Activity of Planterosomal Gel:** *In-vitro* antifungal activity of *G. lucidum* and planterosomal gel was evaluated by agar well diffusion method using *Candida albicans* as a fungal strain. *G. lucidum* and planterosomal gel show moderate antifungal activity with the zone of inhibition of 18.66 and 15.66 mm as compared to the standard (fluconazole) having the zone of inhibition of 31.66 mm. Results of *in-vitro* antifungal activity are shown in **Table 4** and **Fig. 5**.

TABLE 4: *IN-VITRO* ANTIFUNGAL ACTIVITY OF PLANTEROSOMAL GEL AND *G. LUCIDUM*

Name of Compounds	Zone of Inhibition (mm)			
	1st	2nd	3rd	Mean ± S.D.
G. lucidum	20	17	19	18.66 ± 1.5
Planterosomal gel	16	14	17	15.66 ± 1.52
Fluconazole	33	30	32	31.66 ± 1.52
Control	…	…	…	…



G. lucidum **Planterosomal gel** **Fluconazole**

FIG. 5: ANTIFUNGAL ACTIVITY AFTER ADMINISTRATION OF STANDARD AND TEST COMPOUNDS

CONCLUSION: The present research work focused on the development of novel herbal gel formulation based on phytosomal technology because of easy acceptability of herbal formulation, enhanced bioavailability, high efficacy, and less adverse effects. The herbal gel can also be used to treat skin diseases, especially Acne vulgaris [15]. World Health Organization (WHO), as well as our country, has been promoting use of traditional medicine because they are less expensive, easily available and comprehensive, especially in developing countries [16]. The planterosomal gel of *G. lucidum* was formulated for improving retention time of formulation on the skin and enhancing the permeation rate across the stratum corneum. Results of *in-vitro* release study and *ex-vivo* permeation study indicate that planterosomal gel release the active constituents from the formulation in a controlled manner, retard the release rate from the skin surface and consequently increase permeability across stratum corneum.

In-vitro antifungal activity of *G. lucidum* and planterosomal gel was evaluated by agar well diffusion method using *Candida albicans* as a fungal strain. *G. lucidum* and planterosomal gel showed moderate antifungal activity when compared with fluconazole. From the study, it was concluded that the planterosomal gel of *G. lucidum* was effective in the treatment of topical fungal disorders.

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CONFLICT OF INTEREST: We declare that we have no conflicts of interest.

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