FORMULATION AND EVALUATION OF A PHYTOSOMAL GEL CONTAINING AZADIRACHTA INDICA EXTRACT- PHOSPHOLIPID COMPLEX

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ABSTRACT

Aim and objective: The present research aims to develop a phytosomal antifungal gel formulation of Azadirachta indica (neem). The neem phytosomal gel formulation is made for better patient compliance and to reduce the dose of the drug and to avoid first pass metabolism and the other side effects. There are many herbal extracts having excellent in-vitro activity but less penetrating activity through skin because of their macromolecular size and poor lipid solubility, which result in poor absorption and penetration. Method: Thin film hydration technique was used for the formation of phytosome. The phytosome was characterized by particle size, entrapment efficiency, drug content, zeta potential SEM, FTIR. Further, the optimized phytosome complex were formulated as gels using varying polymers in differing concentrations and were assessed for their homogeneity, pH, drug content and in vitro release, ex vivo permeation using Franz diffusion cell. Antifungal activity of phytosome of Azadirachta indica was evaluated on Candida albicans. Results: The particle size, zeta potential, entrapment efficiency and drug content of optimized phytosomal batch was found to be 186.8 nm, -27.5 mV, 80.15%, 88.61 %. The optimized phytosomal gel batch showed the good appearance, pH 6.08, drug content 84.67%. The drug release profile for optimized batch showed invitro release of 88.77% and ex vivo release of 54.9%. The phytosomal gel formulated using Azadirachta indica of optimized batch showed enhanced skin permeability and antifungal activity. Conclusion: The study demonstrated that the phytosomal gel developed using methanolic extract of A. indica would be beneficial for treating fungal infections.
KEYWORDS: Phytosome, soya lecithin, entrapment, soxhlet, polydispersity index, permeability.

INTRODUCTION
Phytosomes are used as a new advanced modern dosage formulation technology for herbal drugs and with comparison with conventional herbal extract phytosomes showed improved better absorption and, as a result, produce better results.\[1,2\] The term "phyto" means plant while "some" means cell-like. Phospholipids is an important agent for the development of phytopharmaceuticals due to its biocompatibility with the phytochemicals.\[3\] Phytosomes are generally prepared by adding accurate amount of phospholipid (Soya lecithin) with herbal extracts in a solvent. Soya lecithin is the important constituent which has phosphatidylcholine in it which is having a dual function with lipophilic and hydrophilic nature. The active constituents get attached with the choline part, whereas the phosphatidyl part forms the envelope around the choline and phytocoentituents complex. It results in the formation of lipid complex with better stability and bioavailability.\[4\] Entrapment of the compounds and the phospholipid shows the formation of hydrogen bond with benefits of high entrapment efficiency, better stability profile therefore resulting in enhanced bioavailability and greater efficacy\[5–8\]. Phytosomes has a functional importance related to skin and its delivery through skin as it improves the absorption of phytoconstituents through skin, it regulates the physiology of skin compositions, and the improvement in the functioning of skin.\[9\] Neem (Azadirachta indica) is an evergreen tree that belongs to the Meliaceae family and is found throughout world. It is also known as margosa or Indian lilac.\[10\] For the treatment of the fungal infection and by survey, the methanolic extract of neem was reported to have in vitro antimicrobial and antifungal activities against S. aureus, E. coli, Ps. aeruginosa and C. albicans.\[11\] Fungal infection of the skin is now a day's one of the common dermatological problem. There are wide choices for treatment for fungal infections from solid dosage, semisolid dosage form and liquid dosage formulation. For the topical formulation, gels have widely accepted in cosmetics and pharmaceuticals. Within the major group of semisolid preparations, the use of gels has expanded both cosmetics and pharmaceutical preparations.\[12\] The key objective of the present study is to develop the nano vesicles phytosomal gel of methanolic extract of Azadirachta indica by specific method and evaluate its antifungal activity.
MATERIAL AND METHODS

Materials

*Azadirachta indica* leaves were procured from local market, Mumbai, India. Soya lecithin and Cholesterol was purchased from Vishal Chemicals, Mumbai, India. Carbopol 934 was purchased from Vishal Chemicals, Mumbai, India. All other chemicals and reagents used were of analytical grade.

Methods

Preparation of the extract

The fresh leaves (plant authentication specimen number 1797) were shade dried for 10 days and were crushed directly by grinder. The freshly collected leaves were thoroughly washed with distilled water, shade dried, powdered were extracted using solvents such as methanol, ethanol, ethyl acetate, water and chloroform separately using Soxhlet apparatus. Methanolic extract of *Azadirachta indica* powder was obtained after soxhlet extraction. The extract was further placed in a porcelain dish on an electric water bath at 40 °C to remove solvent. Weight of the extract was calculated and preliminary phytochemical screening was carried out.

PREPARATION OF PHYTOSOME

Phytosomes were prepared by using thin layer hydration method with different molar ratio of phospholipid and *Azadirachta indica* extract with slight modifications. Cholesterol and soya lecithin were taken in round bottom flask and both were dissolved by addition of 1:1 in ratio of chloroform and methanol. The solution was rotated at 200rpm at 60°C ± 2°C, until all the organic phase evaporated and a thin layer was formed on the wall of the round bottom flask. The crude extract of neem was dissolved in 20 ml of distilled water in a separate beaker and it was hydrated with the thin film of cholesterol and soya-lecithin in the round bottom flask and this mixture again rotated at 60°C ± 2°C for 1 hour at 200 rpm. Different batches with varying concentration were formulated. The phytosome vesicle containing Neem were subsequently formed, further subjected to sonication or homogenized for 15 minutes for further evaluation and formulation.

CHARACTERIZATION OF PHYTOSOME

1. Physical appearance

The phytosomal batches were evaluated based on the observation with the physical appearance.
2. **Particle size and polydispersity index**

The particle size of *Azadirachta indica* phytosomes was measured by particle size analyzer (Malvern Zeta Sizer, Malvern Instruments, Malvern), with polydispersity index at 25°C and a scattering angle of 90°. The Azadirachta indica phytosomes was diluted with distilled water prior to analysis.

3. **Zeta potential**

Zeta potential was measured for the physical stability of phytosomes. There is a greater stability in the particle if there is a higher electrostatic repulsion between the particles. Zeta potential of the phytosome vesicles for the optimized formulation was determined by using Malvern zeta sizer.

4. **Entrapment efficiency**

The entrapment efficiency of the Neem phytosome suspension was analysed by UV spectrophotometry. The neem phytosomal formulation was centrifuged at 15,000 rpm at 4°C for 30 minutes. Supernatants were collected and the amount of free drug was determined at 281nm using a UV spectrophotometer. The entrapment is calculated by the given formula.\[^{17}\]

\[
\text{Entrapment efficiency (\%) = } \frac{\text{(Total drug)} - \text{(Free drug)}}{\text{(Total drug)}} \times 100
\]

5. **Drug content**

Phytosomes with 10 mg of drug was weighed and taken into a 100 ml volumetric flask. The contents of the flask were dissolved in small quantity of ethanol and sonicated for 30 minutes. Volume was adjusted to 100 ml with ethanol. Contents of the flask were filtered and drug content was determined spectrophotometrically using UV spectrophotometer at 281nm after appropriate dilutions.\[^{18}\]

6. **FTIR**

The compatibility studies were carried out at room temperature using FTIR spectroscopy to determine the drug-drug interaction, drug-excipients interactions used in the formulation. *Azadirachta indica* extract, phospholipid, cholesterol and phytosomal complex were subjected to FTIR spectroscopy and interaction was observed by matching peak matching method.

7. **SEM**

Scanning Electron Microscopy was used to determine particle size distribution and surface
morphology of the complex. Samples were studied using scanning microscope. Then the particle size of the formulation was viewed and photographed using a Scanning electron microscope.[19-20]

8. Stability study

The phytosomal formulation batches were kept for stability study at 25°C ± 0.5°C for 3 months. Further the entrapment efficiency and drug content were evaluated to know the stability of the formulation.

PREPARATION OF PHYTOSOMAL GEL

Preparation of gel: Carbopol 934 was used as gel base by separately dispersing it in distilled water with constant stirring at a moderate speed using magnetic stirrer. The pH of all the formulations was adjusted to 5.5 - 6.5 using triethanolamine. Methyl paraben was also added in to the base.

Incorporation of Phytosomal complex into the gel

The solution of phytosome complex prepared in another beaker was added to the Carbopol base. Different formulations of 1%, 1.5%, 2.5% were prepared using varying concentration of gelling agent. Prepared gels were stored in suitable containers at room temperature for further studies.[19] Further the Azadirachta indica phytosomal gels (APG) were evaluated with the standard extract (AEG) and marketed gel (AMG).

CHARACTERIZATION OF PHYTOSOMAL GEL

1. Homogeneity

The formulated gels were evaluated by visual inspection after the gels have been set in the container. All the batches were tested for their appearance and presence of any aggregates.

2. pH

The pH of various gel formulations was determined using Digital pH meter (Systronics, model 335). pH of the formulations was measured by dipping the electrode completely in the Phytosomal gel, standard extract and marketed neem gel so as to cover the electrode and read out the pH.

3. Viscosity

Viscosity of the formulations was studied using a Brookfield DV-E digital viscometer (Brookfield Engineering Laboratories, Middleboro, MA), with spindle S-64 on 100 rpm.
4. **Spreadability**

Spreadability is the area to which the gel spreads easily on application on any affected area of skin. 1gm of gel was placed on a glass slide and later second slide was placed varying the weights. The time taken for the separation of two slide and the distance by it was calculated.

\[
\text{Spreadability} = \frac{\text{Weight tied to the upper side} \times \text{length of the glass slides}}{\text{Time taken in seconds}}
\]

5. **Drug content**

For the determination of drug content, 1 g of phytosomal gel was completely dissolved in 10 ml of ethanol. Further, the solution was diluted to 10 ml volumetric flask and the absorbance was measured using UV Spectrophotometer against a blank at 281nm.

6. **In-vitro drug release**

The in-vitro drug release study was carried for the phytosomal gel, marketed formulation and standard extract gel was placed on the donor compartment with cellophane membrane immersed into 17 ml of dissolution medium of phosphate buffer of pH 7.4 medium. For in-vitro release temperature was maintained 37°C ± 0.5°C at 150 rpm. 2 ml samples were withdrawn at an interval of 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 hours and replaced with equivalent amount of the PBS medium. The samples were analysed by UV at 281 nm. The amount of drug released was calculated and the percentage drug released was plotted against time.[21]

7. **Ex-vivo drug permeation**

The ex vivo permeation study of the phytosomal gel was performed by taking the goat skin from the nearest slaughter for the studies. Skin was cleaned with cold tap water and the non-dermatome skin was removed with the help of a scalpel. The apparatus used was Franz diffusion cell for the skin permeation studies. The receptor compartment was filled with 17 ml of phosphate buffer of pH 7.4, as a dissolution medium and stirred with a magnetic bead. The temperature was maintained at 37±1°C with a speed of 150 rpm. The skin was placed with the stratum corneum facing the donor compartment of the Franz diffusion cell. Formulation of *Azadirachta indica* phytosomal gel was placed on the donor compartment. At predetermined time intervals of 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8 hours 2ml of sample was taken from the receptor compartment and an equivalent amount of phosphate buffer solution was added to maintain the sink condition.
8. **Skin retention/deposition studies**
For the skin deposition study the excess formulation was washed and the remaining skin was cut into small pieces to determine the amount of drug in the viable skin which is epidermis and dermis. Viable part of the skin was taken in a tube containing phosphate buffer solution (pH 7.4) and further sonicated and then centrifuged to extract the drug for estimation. The supernatant was subsequently filtered through a 0.45-μm filter and was analysed by UV at 281nm.[22]

9. **Antifungal study**
The *in-vitro* antifungal activity was evaluated by using agar well diffusion method by taking the standard extract. Fungi *Candida albicans* (ATCC 10231) was selected for the activity where the Sabouraud’s dextrose agar was used as the medium for preliminary antifungal activity. The medium was prepared by dissolving in water and autoclaving at 121°C for 15 minutes. Acidic medium favours the growth but excess of acid prevents solidification of agar.

The sterile hot Sabouraud’s agar medium was poured in each clean petri plate and allowed to harden for some time. A spreader was used in the adjusted inoculums suspension of fungi *Candida albicans* (ATCC 10231) and streaked to cover the entire surfaces of Sabouraud’s agar plates. The flamed sterile borer was used and the medium was bored in which the prepared sample was poured to each bore.[23] This procedure was carried out for the Standard, Phytosome complex batches. The above method was carried out in aseptic condition. These plates were incubated at 28°C for 24 hr. Zone of inhibition were calculated and recorded.

10. **Stability study**
Stability was performed to observe the changes in pH, viscosity, drug content of the optimized phytosomal gel. The formulations were stored at a temperature of 4°C ± 0.5°C and 25°C ± 0.5°C for a period of 3 months. The results were further evaluated for the optimized batch.

**RESULTS AND DISCUSSION**

**Preparation of extract**

Methanolic extract was selected based on the phytochemical testing and the antifungal activity of neem extract with methanol as the solvent.

**Preparation of phytosomes**

*Azadirachta indica* phytosomal formulation was prepared with ten different batches with
varying concentrations of soya lecithin, cholesterol and drug. From these ten batches two batches B5 and B6 were selected as shown in table 1. The optimized batch was selected with parameters showing thin uniform film with 1:1 (5 ml: 5 ml) for the amount of methanol and chloroform, 0.25% soya lecithin and 0.075% cholesterol, water as hydration solvent with high entrapment efficiency, 60°C ± 2°C temperature showed spherical vesicles formed, 15 minutes homogenization showed vesicle size in the range.

CHARACTERIZATION OF PHYTOSOMAL COMPLEX

Particle size and polydispersity index
The average particle size of B5 was found to be 186.8 nm and the range was between 150-200 nm as shown in fig 1. The particle size of B6 batch was found to be 1059.8 nm. The polydispersity index was found to be 0.325 indicating uniformity of B5 batch and 0.602 of B6 batch. The smaller particle is due to the method of preparation, the concentration used of the phospholipid, rpm used for the formulation and the homogenization technique for the size reduction. The smaller the particle size will enhance more the drug penetration through skin and show its activity at the site.

Zeta potential
Zeta potential of the vesicles, is a parameter which is a determinant of stability of vesicular systems. The zeta potential of B5 batch was found to be -27.5 mV as shown in fig 1 indicating good stability of the formulation whereas the zeta potential of B6 was -15.7 mV. The stability is due to the cholesterol in the formulation which acts as a stabilizer. The zeta potential shows the stability without any aggregation of the particle.

Entrapment efficiency
The entrapment efficiency was found to be in the range of 60 to 80 %. The entrapment of B5 was found to be 80.15%. With further increase in the lipid concentration and changing the lipid the entrapment efficiency was decreased and thus it was indicated that the increased amount of lipid doesn’t entrap higher amount of drug as observed in B6 batch with 75.24%. The amount of soya lecithin and cholesterol has an effect on the entrapment and preparation of phytosome. Increasing the amount to some extent or decreasing the amount or ratio may show the decrease in entrapment efficiency.

Drug content
The drug content of *Azadirachta indica* phytosome was found to be in the range of 50 % to 82
% indicating the presence of an acceptable amount of drug in the formulations. The drug content of optimized B5 batch was found to be 88.61% while the B6 batch drug content was found to be 65.84%.

Based on the evaluation and the results from both the batches of B5 and B6 as shown in table 1 it was observed that the B5 batch with 0.25% soya lecithin, 0.075% cholesterol with 15 minutes homogenization with 1:1 ml solvent, the batch was selected as the optimized batch with acceptable results of particle size, polydispersity index, zeta potential, entrapment efficiency and drug content and the results within the range and limit.

Table 1: Optimized batch of *Azadirachta indica* phytosome complex.

<table>
<thead>
<tr>
<th>Batches</th>
<th>Physical Appearance</th>
<th>Particle size (nm)</th>
<th>Polydispersity index</th>
<th>Zeta potential</th>
<th>Entrapment efficiency (%)</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch 5</td>
<td>Stable and without precipitation</td>
<td>186.8 nm</td>
<td>0.325</td>
<td>-27.5 mV</td>
<td>80.15%</td>
<td>88.61%</td>
</tr>
<tr>
<td>Batch 6</td>
<td>Clear solution with precipitation</td>
<td>1059.8 nm</td>
<td>0.602</td>
<td>-15.7 mV</td>
<td>75.24%</td>
<td>65.84%</td>
</tr>
</tbody>
</table>

Fig. 1: Particle size, polydispersity index and zeta potential of optimized batch B5.

**FTIR**

The compatibility between the *Azadirachta indica* extract and excipients was evaluated using FTIR as shown in fig 2. There was no disappearance of peaks in the phytosomal complex, which confirmed the absence of any chemical interaction between the drug and lipid.

**SEM**

The morphology of the drug loaded phytosomal complex was determined by SEM (Scanning
electron microscopy). The sample of phytosome when examined indicated that the formulation contained spherical vesicles, uniform without any aggregation. fig. 3 indicated the images of optimized batch B5.

Fig. 2: FT-IR spectra of *Azadirachta indica* (a), soya lecithin (b), cholesterol (c), complex(d).

Fig. 3: Scanning electron microscopy image of optimized batch B5 at different magnifications.
Stability study
The stability of the phytosomal suspension was evaluated based on entrapment efficiency and drug content after 3 months as shown in table 6. The B5 batch was selected as optimized batch as there was no changes with the entrapment efficiency and drug content.

GEL EVALUATION PARAMETERS
The Azadirachta indica phytosomal gel (APG) was prepared with seven different batches with varying concentration of Carbopol 934 (1%, 1.5%, 2.5%). Carbopol 934 with 1.5 % was considered as the optimized concentration for the phytosomal gel. The phytosomal formulation batches APG1, APG4, APG6 showed optimized results with better drug release for the activity as shown in table 2.

Homogeneity
The batches were prepared and evaluated based on their appearance and were found that all the gel formulations showed good appearance and homogeneity. The optimized batch APG1 and APG4 showed the good texture and appearance without any greasiness and grittiness according to the patient compliance.

pH
pH of all formulations was in the range between 5.8 to 6.0 as this is lying in the normal pH range of skin, 3.0–9.0. The optimized formulation APG1 batch pH was found to be 6.08. This indicated that the pH of gel is non irritating to the skin and within the pH range.

Viscosity
Viscosity of the gel was found to be in the range of 3580 to 4015 cp indicating formation of a stable gel structure. The optimized batch APG1 was having viscosity of 3583 cp.

Spreadability
Spreadability is the extent of area to which the gel spreads easily on application to skin. The spreadability of the prepared gel formulations was carried out and it was concluded that all the formulation showed acceptable spreadability. The range of spreadability was found to be 5.2 to 7.0. The optimized batch APG1 spreadability was found to be 7.01.

Drug content
The drug content of the formulated gels was estimated spectrophotometrically at 281nm. The drug content of all the formulation was found to be in the range of 55 % to 85%. Drug content
of the optimized batch APG1 was found to be 84.67%.

Table 2: Rheological and physical parameters of phytosomal gel batches.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>APG1</th>
<th>APG2</th>
<th>APG3</th>
<th>APG4</th>
<th>APG5</th>
<th>APG6</th>
<th>APG7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogeneity</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>pH</td>
<td>6.08</td>
<td>5.96</td>
<td>5.84</td>
<td>6.06</td>
<td>5.80</td>
<td>6.03</td>
<td>5.9</td>
</tr>
<tr>
<td>Viscosity</td>
<td>3583</td>
<td>4011</td>
<td>4016</td>
<td>3587</td>
<td>4640</td>
<td>4010</td>
<td>4630</td>
</tr>
<tr>
<td>Spreadability</td>
<td>7.01</td>
<td>5.4</td>
<td>5.2</td>
<td>6.8</td>
<td>4.3</td>
<td>6.5</td>
<td>5.3</td>
</tr>
<tr>
<td>Drug content</td>
<td>84.67%</td>
<td>52.04%</td>
<td>41.16%</td>
<td>73.79%</td>
<td>19.40%</td>
<td>62.91%</td>
<td>30.28%</td>
</tr>
</tbody>
</table>

In vitro drug release

The in-vitro study of all the formulations were carried out using Franz diffusion cell for the study as described in the procedure. The prepared formulations were subjected to invitro drug release studies. The data obtained indicated that formulation batches showed % cumulative drug release in the range of 70% to 90% as shown in table 3. The results indicated 88.77% drug release from the optimized APG1 batch in 8 hours. The phytosomes of *Azadirachta indica* showed better release profile than the pure drug extract as show in fig 4. The standard extract showed drug release with 95.34% in 6 hours and marketed gel showed 94.32% in 5 hours as immediate release and the formulation batches showed sustained release.

Table 3: In-vitro drug release profile of *Azadirachta indica* phytosomal gel batches and standard.

<table>
<thead>
<tr>
<th>Time (in hours)</th>
<th>APG1</th>
<th>APG2</th>
<th>APG3</th>
<th>APG4</th>
<th>APG5</th>
<th>APG6</th>
<th>APG7</th>
<th>AEG</th>
<th>AMG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>0.142%</td>
<td>0.266</td>
<td>1.08%</td>
<td>0.458%</td>
<td>1.02%</td>
<td>0.96%</td>
<td>0.139%</td>
<td>4.486%</td>
<td>7.25%</td>
</tr>
<tr>
<td>0.5</td>
<td>3.556%</td>
<td>1.17</td>
<td>4.31%</td>
<td>4.13%</td>
<td>2.99%</td>
<td>4.5%</td>
<td>1.856%</td>
<td>11.05%</td>
<td>16.15%</td>
</tr>
<tr>
<td>1</td>
<td>8.553%</td>
<td>7.42</td>
<td>9.43%</td>
<td>10.38%</td>
<td>6.47%</td>
<td>9.43%</td>
<td>5.65%</td>
<td>20.57%</td>
<td>27.94%</td>
</tr>
<tr>
<td>2</td>
<td>15.49%</td>
<td>14.8</td>
<td>16%</td>
<td>17.51%</td>
<td>11.7%</td>
<td>16.3%</td>
<td>12.03%</td>
<td>33.06%</td>
<td>42.44%</td>
</tr>
<tr>
<td>3</td>
<td>24.14%</td>
<td>23.44</td>
<td>24.3%</td>
<td>25.90%</td>
<td>18.6%</td>
<td>25%</td>
<td>19.1%</td>
<td>47.05%</td>
<td>57.76%</td>
</tr>
<tr>
<td>4</td>
<td>34.86%</td>
<td>33.35</td>
<td>33.5%</td>
<td>35.49%</td>
<td>26.9%</td>
<td>34.6%</td>
<td>28.19%</td>
<td>62.31%</td>
<td>74.4%</td>
</tr>
<tr>
<td>5</td>
<td>46.53%</td>
<td>44.01</td>
<td>43.8%</td>
<td>46.40%</td>
<td>37.4%</td>
<td>45.2%</td>
<td>38.22%</td>
<td>78.58%</td>
<td>94.32%</td>
</tr>
<tr>
<td>6</td>
<td>60.71%</td>
<td>56.37</td>
<td>55.1%</td>
<td>59.39%</td>
<td>48.4%</td>
<td>57.3%</td>
<td>49.69%</td>
<td>95.34%</td>
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<td>7</td>
<td>74.52%</td>
<td>68.54</td>
<td>66.2%</td>
<td>72.57%</td>
<td>59.8%</td>
<td>69.1%</td>
<td>61.3%</td>
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<td></td>
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<tr>
<td>8</td>
<td>88.77%</td>
<td>80.77</td>
<td>77.3%</td>
<td>85.81%</td>
<td>71.4%</td>
<td>81.3%</td>
<td>73.09%</td>
<td></td>
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</tr>
</tbody>
</table>
**Fig. 4:** Comparative invitro drug release profile of different formulations of *Azadirachta indica* phytosome, standard extract and marketed gel.

**Ex vivo drug permeation**

The drug permeation was carried using Franz diffusion cell and the permeation profile was plotted between % cumulative drug permeated v/s time as shown in fig 5. It was observed that the batch APG1 and APG4 batch showed release of 54.9% and 50.49% over standard pure extract showed 18.88% drug release and the marketed gel showed 20.33% in 8 hours as shown in table 4. The flux was highest of the APG1 and APG4 batches with 0.1524 mg/cm²/h and 0.139 mg/cm²/h as compared to the marketed and standard extract gel with 0.0477mg/cm²/h and 0.0542 mg/cm²/h. Permeability coefficient was also highest in the results of the batches APG1 and APG4 with 0.01524 cm²/h and 0.0139 cm²/h whereas of the marketed and the standard extract gel was 0.00477 cm²/h and 0.00542 cm²/h. This indicates that the phytosomal batches shows highest permeation through the skin through topical drug delivery.

**Table 4: Batches with % cumulative release, flux and permeability coefficient.**

<table>
<thead>
<tr>
<th>Batches</th>
<th>% Cumulative drug permeated</th>
<th>Flux (mg/cm²/h)</th>
<th>Permeability coefficient (cm²/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APG1</td>
<td>54.9%</td>
<td>0.1524</td>
<td>0.01524</td>
</tr>
<tr>
<td>APG4</td>
<td>50.49%</td>
<td>0.139</td>
<td>0.0139</td>
</tr>
<tr>
<td>AEG</td>
<td>18.88%</td>
<td>0.0477</td>
<td>0.00477</td>
</tr>
<tr>
<td>AMG</td>
<td>20.33%</td>
<td>0.0542</td>
<td>0.00542</td>
</tr>
</tbody>
</table>
Fig. 6: Comparative ex vivo drug release profile of different formulations of \textit{Azadirachta indica} phytosome, standard extract and marketed gel.

**Skin retention/deposition studies**

The deposition or retention of drug in the skin was evaluated after the ex-vivo permeability studies. The drug retention after 8 hours into the skin was found to be 0.74 mg/cm$^2$ of the optimized batch APG 1. The amount of drug retained in the skin of phytosomal gel is higher than that of the standard gel batch which is 0.20 mg/cm$^2$. The higher permeability and retention of formulation gel batches showed the effective results and release.

**Antifungal study**

Antifungal activity of \textit{Azadirachta indica} against the species Candida albicans (ATCC 10231) was found with the results that the phytosome complex B5 showed 39 mm zone of inhibition as compared to 31mm and 23mm zone of inhibition of phytosome complex B6 and standard after 24 hours as shown in table 5 and fig 6. The phytosome complex of \textit{Azadirachta indica} B5 showed better antifungal activity as compared to phytosome complex B6 and standard. This effective antifungal activity may be due to penetration of vesicle containing plant constituent.

**Table 5: Antifungal study of the optimized batches with zone of inhibition.**

<table>
<thead>
<tr>
<th>Batches</th>
<th>Species</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A (Batch 5)</td>
<td>\textit{Candida albicans} (ATCC 10231)</td>
<td>39 mm</td>
</tr>
<tr>
<td>Sample B (Batch 6)</td>
<td>\textit{Candida albicans} (ATCC 10231)</td>
<td>31 mm</td>
</tr>
<tr>
<td>Standard</td>
<td>\textit{Candida albicans} (ATCC 10231)</td>
<td>23 mm</td>
</tr>
</tbody>
</table>
Stability study

It was found that, parameters studied for the stability study of phytosomal gel were in limits as shown in table. 6 and this indicates that the APG1 was optimized and remain stable during 3 months without any changes or slight changes. The physical appearance does not show any significant changes on storage.

Table 6: Stability study of phytosomal suspension and phytosomal gel of the optimized batches.

<table>
<thead>
<tr>
<th>Time (Months)</th>
<th>Phytosomal Suspension</th>
<th>Phytosomal Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Entrapment efficiency %</td>
<td>Drug content %</td>
</tr>
<tr>
<td></td>
<td>Batch B5</td>
<td>Batch B5</td>
</tr>
<tr>
<td>Initial</td>
<td>80.15 %</td>
<td>88.61 %</td>
</tr>
<tr>
<td>3 months</td>
<td>80.11%</td>
<td>88.57%</td>
</tr>
</tbody>
</table>

CONCLUSION

The novel drug delivery of herbal drugs is a new way for phytoconstituents of plant particularly containing flavonoids and poly phenolic compounds. However, due to its poor lipid solubility and larger molecular size limits the delivery resulting in poor bioavailability. Phytosome one of the drug delivery systems shows potential and advantageous effects and results. The properties of Phytosome with high stability, high carrier capacity, and sustained drug release action up to 8 hours shows the enhanced drug bioavailability and enhanced therapeutic effect, reduction of dosing frequency. The complexation of phytoconstituents and phospholipids shows effective results due to lipophilic nature of phospholipid and offering the herbal drugs with sufficient lipid penetrability. The optimized batch showed acceptable and enhanced results in entrapment drug content drug release profile by invitro and ex vivo study. The phytosomal
batch showed the effective antifungal activity. The phytosomal formulation of *Azadirachta indica* showed effective and enhanced results with enhanced drug delivery as a novel formulation for herbal drugs.

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