Formulation and Performance Evaluation of *Berberis Aristata* Extract Loaded Ethosomal Gel

Nimisha¹, Zeeshan Fatima¹, Chanchal Deep Kaur²

¹Department of Pharmaceutics, Amity Institute of Pharmacy, Amity University, Lucknow Campus, Uttar Pradesh, India, ²Department of Pharmaceutical sciences, Shri Rawatpurwa Sarkar Institute of Pharmacy, Kumhari, Durg, Chhattisgarh, India

Abstract

Aims: The aim of the present research work is to prepare ethosome of standardized *Berberis aristata* extract and to evaluate for vesicle shape, size, polydispersity index (PDI), zeta potential (ZP), entrapment efficiency (EE), and in vitro permeation studies. **Materials and Methods:** Ethosomes were prepared using soayphosphatidylcholine (1-3%) and ethanol (10-40%) by modified cold method and were characterized. **Results:** The size of ethosomes were found to be in the range of 110.98-357.34 nm while PDI ranges from 0.114 to 1.56 and ZP was between −20.0 and −39.4 mV. Morphology studies showed unilamellar structure under transmission electron microscope and phase contrast microscope showed smooth surface. The EE of ethosomes was found to be in the range of 48.05% to 92.08%. Ethosomes were further added to carbopel 934P for gel formation, and subsequently, evaluated for their physicochemical properties. **Discussion:** In vitro diffusion study was conducted for ethosomal dispersion, hydroethanolic solution, ethosomes incorporated in viscous gel, and conventional gel using Franz diffusion cell for the biomarker compound berberine. **Conclusions:** It can be concluded that *B. aristata* loaded ethosomal delivery system is an encouraging approach for herbal drugs.

Key words: Dermatitis, ethanol, ethosomes, extract, gel, soyaphosphatidylcholine

INTRODUCTION

*Berberis aristata*, belongs to the family Berberidaceae, is an erect spiny shrub which is hard and yellow. It is majorly found growing in the sub-Himalayan regions and Nilgiri Hills of Southern India. The plant contains a number of important phytochemicals which includes alkaloids such as protoberberine, isoquinoline, bisbenzylisoquinoline, flavonoids, and phenolic acids.

The application of *B. aristata* extract as a potential topical drug candidate for dermatological disorders can be explored by incorporating it into nanovesicles like ethosomes. Ethosomes are novel vesicular carrier which can permeate intact through the human skin due to its high elasticity properties and can improve the dermal pharmacological action.[¹] They have the ability to overcome the problems associated with conventional formulation of plant extracts like poor absorption profile due to macromolecular size and low lipid solubility. Till today, none of the existing literature highlights the conversion of this extract into novel vesicular delivery system like ethosomes and its characterization thereof.

In this study, ethosomes have been formulated of standardized *B. aristata* extract (roots, ethanolic 70% v/v) and evaluated for vesicle shape, size, polydispersity index (PDI), zeta potential (ZP), entrapment efficiency (EE), and in vitro permeation studies. The quantification and standardization of *B. aristata* extract have been carried out using its alkaloidal content as biomarker.[²,³]

Corresponding Author:

Dr. Zeeshan Fatima, Amity Institute of Pharmacy, Amity University, Uttar Pradesh, Lucknow Campus, Uttar Pradesh, India. Phone:+91-9415335922.
E-mail: zfatima@amity.edu

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MATERIALS AND METHODS

Material

Standardized *B. aristata* extract (ethanolic 70% v/v) was obtained as a gift sample from Aushadhi Herbals New Delhi, India. Berberine chloride dihydrate was obtained as a gift sample from Natural Remedies Pvt. Ltd., Bengaluru. All other solvents and reagents used were of analytical grade.

Preformulation studies of *B. aristata* extract

Preformulation studies of *B. aristata* extract were performed [Table 1].

High-performance thin layer chromatography (HPTLC) of *B. aristata* extract

Precoated silica gel 60 F<sub>254</sub> aluminum plates of thickness 0.2 mm were used. The mobile phase n-propanol:water:formic acid (8.0:1.8:0.2) was taken. Standard solutions of 2, 4, 6, 8 and 10 µl (berberine) and test solution (*B. aristata*) extract of 10 µl was applied in the form of band with the help of lintomas 5 applicator attached to CAMAG 5 HPTLC system which was programmed through WINCATS software version 1.4.8.

The air-dried plates were visualized in ultraviolet (UV) radiation at 254 and 360 nm. The chromatograms were scanned by densitometer at 347 nm. The Rf value and fingerprint data were recorded by WINCATS software version 1.4.8.

Drug-excipient compatibility

The extract was mixed with soyaphosphatidylcholine (SPC) in 1:1 ratio and placed in glass vials properly capped and sealed for drug excipient compatibility study. The powder mixture was analyzed using Fourier transform infrared (Agilent Cary 630).

Preparation of ethosomes

Ethosomes were prepared by slight modification of cold method using varying concentrations of SPC (1-3%) and ethanol (10-40%) [Table 2]. Initially, SPC was taken and dissolved in ethanol in a completely closed flask at 30°C. To this solution, 10 mg of the extract dissolved in hot distilled water (30°C) was added slowly using syringe and the whole system was stirred for 15 min at 900 rpm. Finally, the formulations were sonicated for 5 min and then refrigerated.

Characterization of ethosomes

ZP, vesicle size and PDI were measured by zetasizer (Malvern Instruments, Malvern).<sup>[4,6]</sup>

Entrapment efficiency

Aliquots of ethosomal dispersions were subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm. The clear supernatant was siphoned off carefully to separate the unentrapped extract. Sediment was treated with 1 ml of 0.1% Triton X-100 to lyse the vesicles, and then diluted with phosphate buffer saline (PBS) (pH 7.4). The EE was determined in terms of percentage berberine (BE) content in the sediment.

Since berberine is one of the major and important alkaloids found in *B. aristata* which is responsible for its pharmacological property; therefore, it was considered as a biomarker for estimations.<sup>[2,3,7,8]</sup>

The percent entrapment was calculated using the formula.<sup>[9]</sup>

\[
\text{%Entrapment efficiency} = \left( \frac{\text{amount of BE in sediment}}{\text{amount of BE in the extract added to ethosome}} \right) \times 100
\]

Visualization of vesicles by transmission electron microscope (TEM) and phase contrast microscope

All the prepared batches were observed under phase contrast microscopy with the magnification power of ×100 (Olympus). Photographs of vesicles were taken using Olympus camera (Olympus MJU 9010). Optimized batch was dispersed in distilled water, and 10 µl of diluted dispersion was placed on carbon-coated grid. It was visualized using Jeol/JM 2100, source LaB6 electron microscope (TEM) with an accelerating voltage of 200 kV for surface appearance and shape.

Table 1: Results of preformulation studies of *B. aristata* extract

<table>
<thead>
<tr>
<th>UV spectrophotometry</th>
<th>Ethanol water</th>
<th>PBS 7.4</th>
<th>Distt water</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Absorption maxima</strong></td>
<td>340 nm</td>
<td>203 nm</td>
<td>275 nm</td>
</tr>
<tr>
<td><strong>Beer's Law limit</strong></td>
<td>5-25 µg/ml</td>
<td>5-25 µg/ml</td>
<td>5-25 µg/ml</td>
</tr>
<tr>
<td><strong>Regression equation</strong></td>
<td>y=0.0037x−0.0012</td>
<td>y=0.008x−0.008</td>
<td></td>
</tr>
<tr>
<td><strong>Intercept</strong></td>
<td>−0.0012</td>
<td>0.008</td>
<td>0.031</td>
</tr>
<tr>
<td><strong>Slope</strong></td>
<td>0.0037</td>
<td>0.008</td>
<td>0.042</td>
</tr>
</tbody>
</table>

Incorporation into hydrophilic gels

Among the 12 ethosomal batches, F 12 batch showed highest EE, optimum PDI and ZP which was further incorporated into carboxyvinyl polymer carbomer (Carbopol 934P) gel formulations [Table 3 and Figure 1a and b]. Carbopol 934P (1.5% w/w) was soaked in a minimum amount of water for an hour followed by addition of 10 ml of ethosomal dispersion containing B. aristata extract (10 mg). It was then stirred continuously at 700 rpm in a closed vessel whose temperature was maintained at 30°C until homogeneous ethosomal gels (GELF 12) was achieved. To maintain the neutral pH triethanolamine was added slowly to the stirring mixture.[10]

In vitro diffusion studies

In vitro diffusion study was performed for hydroalcoholic solution (HA) solution, ethosomal dispersion (F 12), ethosomal gel formulation (GELF 12), and conventional gel formulation using dialysis membrane (Hi media). It was placed in PBS (7.4) for 6 h to attain saturation before starting permeation study and then mounted between the donor and receptor compartment of the Franz diffusion cell (fabricated with glass, the surface area available for diffusion was 2.54 cm²). The release rate of B. aristata was analyzed by placing the required sample in the donor cell compartment. To prevent contamination and evaporation, the donor compartment was covered with parafilm. The receptor chamber was filled with PBS (7.4) and was maintained at 37°C with continuous stirring. 1 ml aliquot of receptor phase solution was withdrawn at time intervals of 0.5, 1, 2, 4, 8 and 24 h, and the same volume of fresh medium was added back into the chamber. The quantification was done using UV spectrophotometer (ShimadzuModel No. 1800) at 203 nm. Cumulative amount of drug permeated per unit area versus time graph was plotted, and transdermal flux (J) was calculated from the slope of linear portion [Figure 2].[11-14]

Permeation data analysis

To study the release rate profile, the data obtained from in vitro drug release study were fitted in different kinetic equations: Zero order as the cumulative percent of drug remaining versus time, first order as the log cumulative percentage of drug remaining versus time, Higuchi’s model as the cumulative percent drug remaining versus square root of time, Hixson Crowell cube root model and Korsmeyer-Peppas model as the log cumulative percentage of drug released versus log time.

Viscosity and pH measurement

Viscosity of ethosomal gel formulation was measured using Brookfield viscometer (Model No DV-III ULTRA) using spindle no 06 at 100 rpm, and pH measurements of the formulations were done using digital pH meter (RI-152-R).

Table 2: Composition of various B. aristata extract loaded ethosomal batches

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>% SPC</th>
<th>Ethanol: Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1</td>
<td>10:90</td>
</tr>
<tr>
<td>F2</td>
<td>1</td>
<td>20:80</td>
</tr>
<tr>
<td>F3</td>
<td>1</td>
<td>30:70</td>
</tr>
<tr>
<td>F4</td>
<td>1</td>
<td>40:60</td>
</tr>
<tr>
<td>F5</td>
<td>2</td>
<td>10:90</td>
</tr>
<tr>
<td>F6</td>
<td>2</td>
<td>20:80</td>
</tr>
<tr>
<td>F7</td>
<td>2</td>
<td>30:70</td>
</tr>
<tr>
<td>F8</td>
<td>2</td>
<td>40:60</td>
</tr>
<tr>
<td>F9</td>
<td>3</td>
<td>10:90</td>
</tr>
<tr>
<td>F10</td>
<td>3</td>
<td>20:80</td>
</tr>
<tr>
<td>F11</td>
<td>3</td>
<td>30:70</td>
</tr>
<tr>
<td>F12</td>
<td>3</td>
<td>40:60</td>
</tr>
</tbody>
</table>

SPC: Soyaphosphatidylcholine, B. aristata: Berberis aristata

Table 3: Entrapment efficiency, vesicle size polydispersity index and zeta potential of ethosomal batches

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Entrapment efficiency (%)</th>
<th>Vesicle size (nanometers)</th>
<th>PDI</th>
<th>Zeta potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>48.05±1.73</td>
<td>220.53±13</td>
<td>0.884±0.052</td>
<td>-38.8</td>
</tr>
<tr>
<td>F2</td>
<td>59.18±1.14</td>
<td>135.57±14</td>
<td>0.963±0.038</td>
<td>-37.7</td>
</tr>
<tr>
<td>F3</td>
<td>65.04±0.70</td>
<td>110.98±15</td>
<td>1.56±0.042</td>
<td>-31.2</td>
</tr>
<tr>
<td>F4</td>
<td>70.16±2.00</td>
<td>97.54±12</td>
<td>0.741±0.016</td>
<td>-39.4</td>
</tr>
<tr>
<td>F5</td>
<td>75.19±1.11</td>
<td>255.65±19</td>
<td>0.295±0.007</td>
<td>-33.4</td>
</tr>
<tr>
<td>F6</td>
<td>70.79±1.14</td>
<td>242.42±12</td>
<td>0.599±0.052</td>
<td>-35.3</td>
</tr>
<tr>
<td>F7</td>
<td>84.64±0.83</td>
<td>210.32±11</td>
<td>0.587±0.003</td>
<td>-44.0</td>
</tr>
<tr>
<td>F8</td>
<td>85.54±2.11</td>
<td>202.06±13</td>
<td>0.066±0.013</td>
<td>-30.6</td>
</tr>
<tr>
<td>F9</td>
<td>83.11±1.83</td>
<td>298.31±14</td>
<td>0.392±0.013</td>
<td>-23.2</td>
</tr>
<tr>
<td>F10</td>
<td>84.69±1.88</td>
<td>271.09±12</td>
<td>0.461±0.017</td>
<td>-20.0</td>
</tr>
<tr>
<td>F11</td>
<td>88.50±0.83</td>
<td>260.34±10</td>
<td>0.554±0.016</td>
<td>-36.9</td>
</tr>
<tr>
<td>F12</td>
<td>92.08±0.64</td>
<td>245.10±11</td>
<td>0.114±0.032</td>
<td>-31.1</td>
</tr>
</tbody>
</table>

PDI: Poly dispersity index, the results are mean±SD (n=3), SD: Standard deviation
Spreading diameter

The spreadability of gel formulation was determined by measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm × 20 cm) after 1 min. The standard weight applied on the upper plate was 125 g.[15]

Drug content of the formed gels

About 500 mg of gel was taken and dissolved in 50 ml of pH 7.4 PBS. The solution was then passed through the filter paper, and 50 µl of the filtrate was withdrawn. The filtrate was diluted by adding 3.5 ml of distilled water, and the drug content was measured spectrophotometrically at 203 nm against corresponding gel concentration.

Statistical analysis

Data are expressed as mean ± standard error of mean. Differences in the in vitro release profile of prepared formulations were tested for significance using independent t-test using SPSS-12.0. Difference was considered significant when P < 0.05. Graphs were prepared using GraphPad Prism 3 (Graph Pad Software, Inc).

RESULTS AND DISCUSSION

Ethosomes of B. aristata extract have been prepared and in vitro permeation has been performed. It has been found that the synergistic effect of phospholipid and higher

Figure 1: (a) The zeta potential of F12 ethosomal formulation, (b) the size and size distribution F12 ethosomal formulation
The IR spectra for drug excipient compatibility study showed major peaks at 3382.4 cm\(^{-1}\), 2926.1 cm\(^{-1}\), 1646.8 cm\(^{-1}\), 1423 cm\(^{-1}\), 1367 cm\(^{-1}\), 1042 cm\(^{-1}\) in pure extract the corresponding peaks were also obtained in the extract excipient mixture with slight shifting. Beside these peaks another distinct peak at 1738 cm\(^{-1}\) was observed indicating the presence of phosphate group in soylecithin. It is evident from the data that the characteristics peaks of extract were not affected in the presence of soylecithin implying that extract and excipient are compatible with each other [Figures 5 and 6].

Overall, performance of transdermal drug delivery system is generally governed by morphology and vesiclular size.
It is evident from the results that the size of the vesicles increased with increasing concentrations of SPC from 1% to 3%, whereas concentration of alcohol affected the vesicle size inversely, i.e., higher concentrations of ethanol produced lower vesicle size. This observation supports the findings, of[16,17] which state that higher concentration of ethanol is responsible for decrease in the size of vesicle as it furnish a surface negative net charge to the vesicular systems by altering some surface characteristics.

Polydispersity index (PDI) was considered for evaluation of homogeneity of prepared ethosomes on the basis of their vesicle size distribution. The PDI value lied between 0.114 and 1.56, inferring that all the batches showed narrow distribution except batches F1, F2 and F3.

ZP is an important parameter that affects stability. All the ethosomal formulation were found to have negative ZP (−20.0 to −39.4 mV) due to the net charge of the lipid composition in the formulation. The negative ZP is responsible for enhanced percutaneous permeation of drug.

Delivery potential of ethosomal system is directly affected by its drug carrying capacity which is determined in terms of EE. The EE of ethosomes was determined for all the formulations. Both, the amount of SPC and ethanol, influenced the entrapment of the herbal extract inside lipid vesicles in a positive way. The ranges of EE of ethosomes were between 48.05% and 92.08%. The ethanol concentration in the ethosome system should not be too high, and generally, should be kept below 45%. As increasing concentration of ethanol results in leaking of the drug from the lipid bilayer due to which EE decreases[9,20] therefore ethanol concentration only up to 40% was considered.

Ethosomal formulation fabricated with 3% SPC and varying ethanol concentration (20%, 30% and 40%) exhibited 83.11%, 88.5% and 92.08% EE, respectively [Table 3]. Varying SPC concentration (1%, 2% and 3%) with fixed amount of ethanol (30%) also showed difference in EE ranging from 66.04% (SPC 1%) to 88.50% (SPC 3%). It can thus be inferred that EE is dependent on the concentration of both ethanol and SPC. Higher EE with increased amount of ethanol is possibly due to increased solubility of B. aristata extract in ethanol present in the ethosomal core. This is in accordance with the previous finding by Paolino et al., 2012.[21]

On the basis of small vesicle size, uniform size distribution, and higher EE batch F 12 was selected for in vitro diffusion studies [Figure 1a and b] and further incorporated into gel formulation (GELF12) for in vivo studies.

Sustained release characteristic is one very important feature of ethosomes.

The cumulative amount of drug release from in vitro diffusion studies was calculated for F12, GELF 12, HA extract solution and conventional gel formulation. Percent cumulative amount
of drug release in 24 h was found to be 78.51%, 79.51%, 38.08% and 29.9% for Batch F12, GELF 12, HA extract solution, and conventional gel formulation, respectively [Figure 8].

Among them, percent drug released from Batch GEL F12 was significantly higher than HA extract solution and conventional gel formulation ($P < 0.05$). Percent cumulative amount of drug release for ethosomes is also significantly higher than conventional marketed formulation ($P < 0.05$). This may be due to the presence of ethanol in ethosomal formulations as compared to conventional gel which is devoid of any ethanol concentration. Ethanol provides the vesicles soft flexible characteristics which allow them to permeate more readily into deeper layers of skin. This is also in accordance with previous findings of Chourasia et al., 2011. [19]

HA drug solution released most of the drug within 2-4 h. Cumulative amount of drug permeated per unit area versus time graph was plotted and transdermal flux (J) was calculated from the slope of linear portion [Figure 2a]. On the analysis of transdermal flux of the same formulations, it was found that results followed the similar pattern as that of cumulative percent drug release [Figure 2b] which varied from 66.69±16.35 µg/cm²/h (conventional gel) to 163.5 µg/cm²/h (GEL F12). It is evident from the results that flux of ethosomal gel was 2.45 times higher than conventional gel and 1.9 times higher than HA extract solution.

The data obtained from in vitro release study was fitted in different kinetic equations to access the release rate profile. Both the batches F12 and GELF 12 were found to have best fit for the Higuchi kinetic model having $r^2$ value of 0.880 for F 12 and $r^2$ 0.931 for GELF12. This implies slow and steady release by the process of diffusion as proposed by Higuchi.

The prepared gels were evaluated for physical appearance, pH, spreadability, viscosity, and drug content. Gels were found to be smooth, homogenous, yellowish white in color, pH lying in the normal skin pH range, easily spreadable, and viscosity ranging between 4500 and 4800 cps [Table 4].

### CONCLUSION

The prepared novel ethosomal system incorporating B. aristata extract have shown enhanced permeation profile as compared to the conventional formulation of B. aristata. We conclude that transdermal delivery of B. aristata extract through ethosomal system may be a better approach for dermatological disorders. However, further biological and clinical research have to be carried out to explore the therapeutic potential of this extract for treating dermatological disorders which will be helpful in the development of safe and efficacious herbal formulations.

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### REFERENCES


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