ORIGINAL ARTICLE

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

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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 soyaphosphatidylcholine (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 carbopol 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, ethanols, ethosomes, extract, gel, soyaphosphatidylcholine

INTRODUCTION

Berberis aristata, belongs to the family Berberidaceae, is an erect spinous 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]

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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_{254} 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).[4-6]

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 ×100 to lyses 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.[2,3,7,8]

The percent entrapment was calculated using the formula.[9]

%Entrapment efficiency = (amount of BE in sediment ÷ amount of BEin the extract added to ethosome)×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

UV spectrophotometry	Ethanol water	PBS 7.4	Distt water
Absorption maxima	340 nm	203 nm	275 nm
Beer's Law limit	5-25 µg/ml	5-25 µg/ml	5-25 µg/ml
Regression equation y=0.042+0.031	y=0.0037×–0.0012	y=0.008×–0.008	
Intercept	–0.0012	0.008	0.031
Slope	0.0037	0.008	0.042
Log P 1.5			

UV: Ultra violet, *B. aristata: Berberis aristata*, PBS: Phosphate buffer saline

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 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 cm^2). 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]

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

Formulation code	% SPC	Ethanol: Water
F1	1	10:90
F2	1	20:80
F3	1	30:70
F4	1	40:60
F5	2	10:90
F6	2	20:80
F7	2	30:70
F8	2	40:60
F9	3	10:90
F10	3	20:80
F11	3	30:70
F12	3	40:60

SPC: Soyaphosphatidylcholine, *B. aristata: Berberis aristata*

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 v*er*sus 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 3: Entrapment efficiency, vesicle size polydispersity index and zeta potential of ethosomal batches

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

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

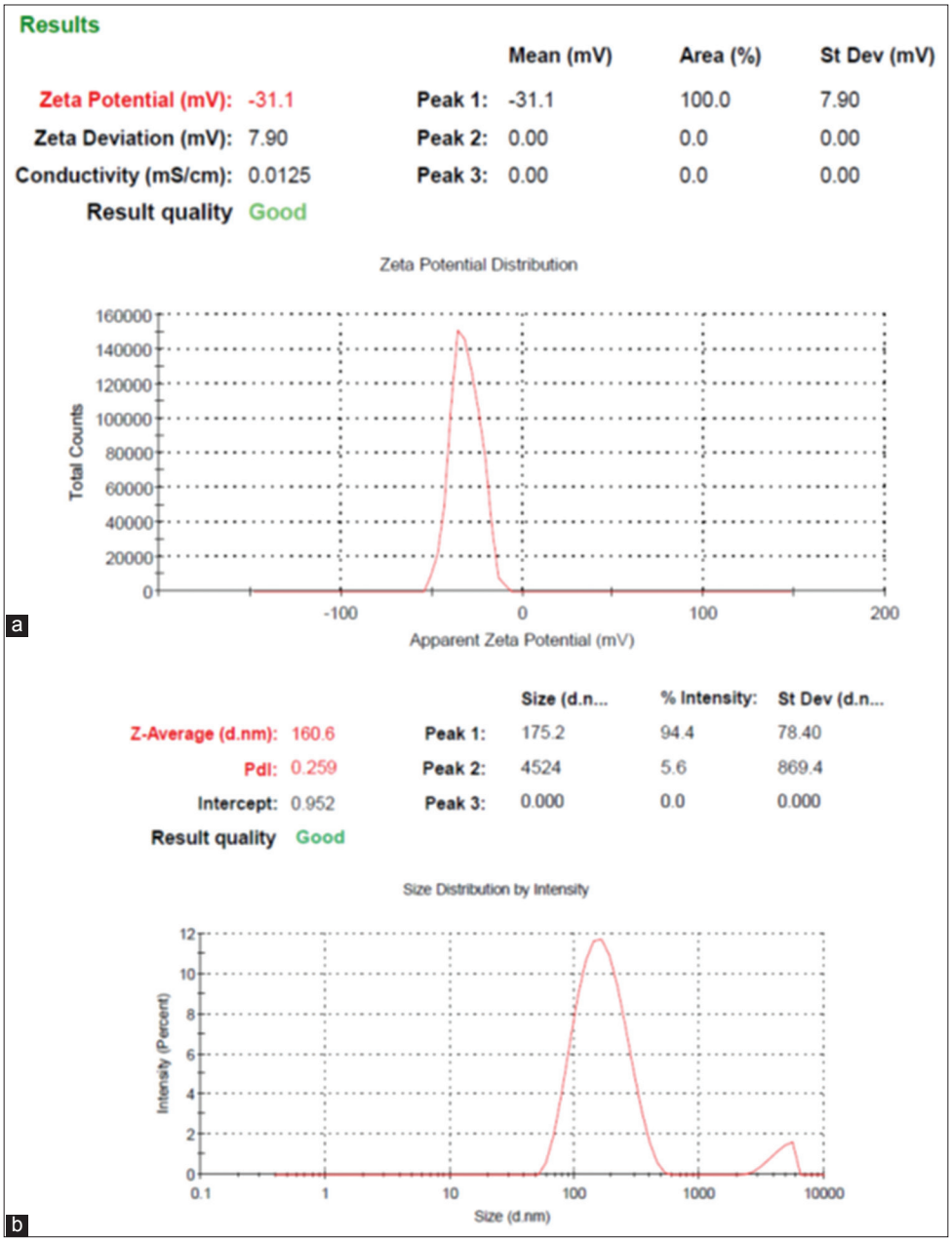

Figure 1: (a) The zeta potential of F12 ethosomal formulation, (b) the size and size distribution F12 ethosomal formulation

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

concentration of alcohol in ethosomes helps the entrapped drug reach the deeper skin layers. The above finding can be corroborated with the study that reports 87 fold increase in the steady state transdermal rate, of trihexyphenidyl hydrochloride ethosomes composed of phospholipid, ethanol, and water as compared with normal liposome.[1]

In the present study, the HPTLC characterization of *B. aristata* was carried out. The peaks obtained in the tracks for extract was analyzed and the Rf value was compared to the standard. The chromatogram of berberine showed Rf value of 0.40. The presence of a specific peak for berberine at Rf around 0.38 was recorded and considered as a positive result for *B. aristata* extract [Figures 3 and 4].

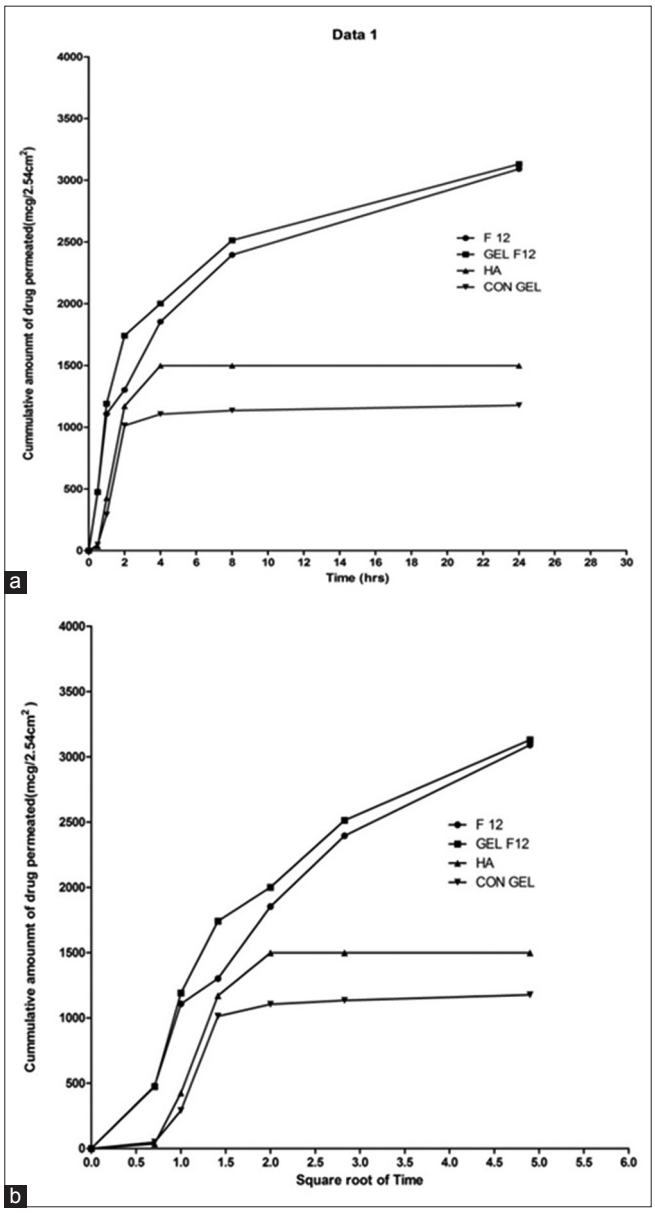

Figure 2: (a) Comparative graph of *in-vitro* permeation of *Berberis aristata* extract from various formulations through 2.54 cm2 values represent mean ± SD (n=3), (b) comparative graph of cumulative amount of drug permeated versus square root of time

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 soyalecithin. It is evident from the data that the characteristics peaks of extract were not affected in the presence of soy lecithin 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.

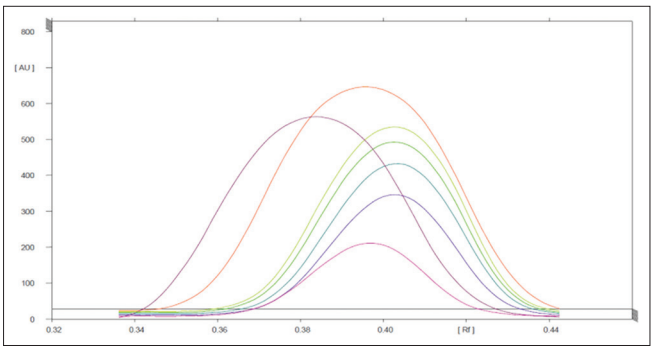

Figure 3: Comparative chromatogram of all tracks of berberine in standard and extract (*Berberis aristata*)

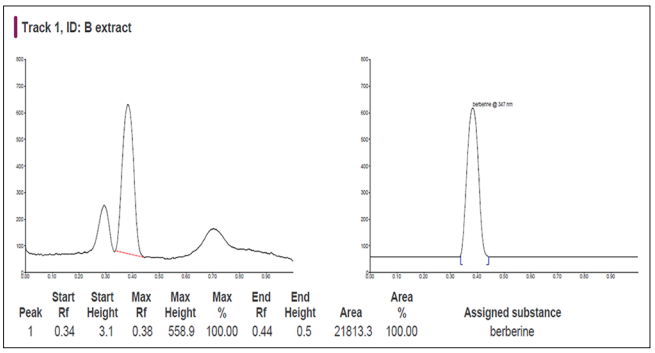

Figure 4: High performance thin layer chromatography chromatogram of *Berberis aristata* extract

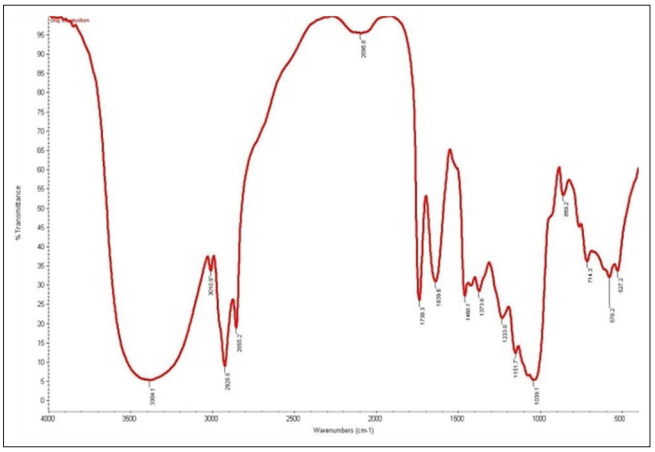

Figure 5: Infrared spectrum of *Berberis aristata* extract

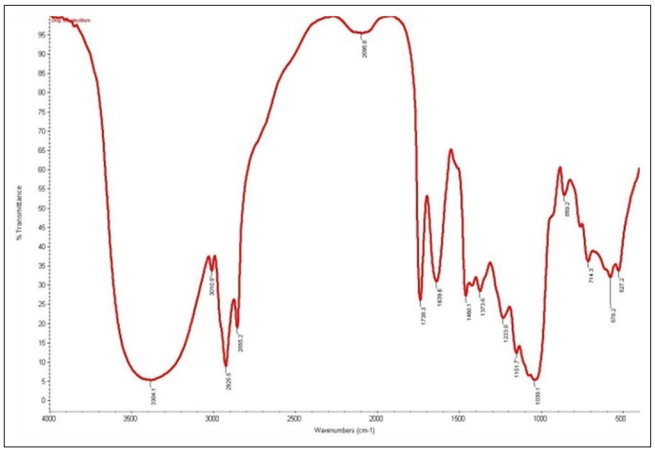

Figure 6: Infrared spectrum of *Berberis aristata* extract and soya lecithin

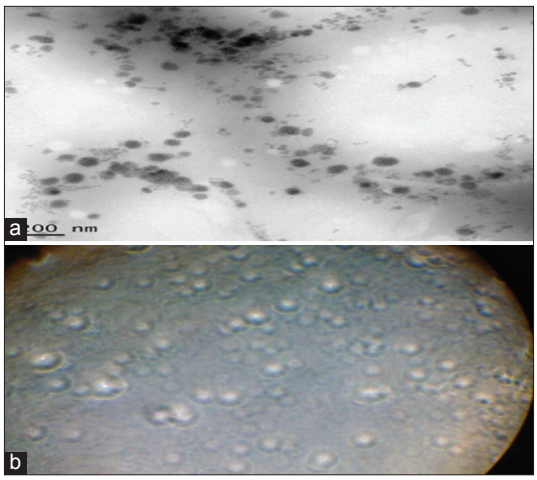

Figure 7: (a) Transmission electron microscope image of F12 ethosomal formulation, (b) phase contrast microscopy image of F12 ethosomal formulation (×10)

Transmission electron micrograph of ethosomal vesicles showed spherical shape with unilamellar structure [Figure 7a]. Phase contrast microscopy also showed the surface morphology of ethosomes [Figure 7b]. All the images depict smooth surface.

It has been reported that if the vesicular size is <300 nm, they can deliver their contents into deeper layers of skin to some extent.[16,17] Size analysis of the formulation depending upon the concentration of SPC and ethanol was found to range from 97.54 to 298.31 nm [Table 3].

Ethosomal dispersions were produced with increasing amounts of ethanol (10 %, 20%, 30%, and 40%) and SPC concentration (1%, 2%, and 3%). In the study, while keeping the concentration of SPC at 1%, it was found that by increasing concentration of ethanol from 10% to 40%, the size of the vesicles decreased from 220.53 to 97.54 nm. In the similar manner, the SPC concentration was taken at 2% and 3% while ethanol was increased from 10% to 40%. It was found that vesicle size decreased from 255.65 to 202.06 and 298.31 to 245.10 nm, respectively [Table 3].

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[18,19] 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

Table 4: Evaluation of physicochemical properties of gel formulations

Formulation code	Color	Homogeneity	Texture	Viscosity (centipoise)	pH	Spreading diameter after 1 min (mm)	Drug content (%)
GELF12	Yellowish Brown	Homogenous	Smooth	4780±0.5	6.2±0.02	55±1.5	46.47±0.80

Values are Mean±Standard deviation (SD) for n=3

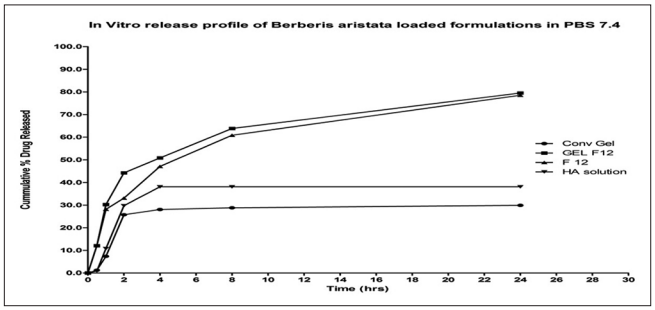

Figure 8: *In vitro* release profile of F12, HA solution, GELF 12, conventional formulation

of drug release in 24 h was found to 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 characterstics which allow then 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 66.69163.5 µg/cm^2/h (conventional gel) to 163.5 µg/cm^2/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 0.931for 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 theurapeutic 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

1. Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCl: Ethosomes vs. Liposomes. Biomaterials 2000;21:1879-85.
2. Kushwaha SK, Kushwaha N, Maurya N, Rai A. Role of markers in the standardization of herbal drugs: A review. Arch Appl Sci Res 2010;2:225-9.
3. Andola HC, Rawal R, Rawat M, Bhatt I, Purohit VK. Analysis of berberine content using HPTLC fingerprinting of root and bark of three Himalayan *Berberis* species. Asian J Biotechnol 2010;2:239-45.
4. Li G, Fan Y, Fan C, Li X, Wang X, Li M, *et al*. Tacrolimus-loaded ethosomes: Physicochemical characterization and *in vivo* evaluation. Eur J Pharm 2012;82:49-57.
5. Zhai Y, Xu R, Wang Y, Liu J, Wang Z, Zhai G. Ethosomes for skin delivery of ropivacaine: Preparation, characterization and *ex vivo* penetration properties. J Liposome Res 2015;25:316-24.

6. Khan NR, Wong TW. Microwave-aided skin drug penetration and retention of 5-fluorouracil-loaded ethosomes. Expert Opin Drug Deliv 2016;13:1209-19.
7. Gupta SK, Agarwal R, Srivastava S, Agarwal P, Agrawal SS, Saxena R, *et al*. The anti-inflammatory effects of *Curcuma longa* and *Berberis aristata* in endotoxin-induced uveitis in rabbits. Invest Ophthalmol Vis Sci 2008;49:4036-40.
8. Saraf S, Jeswani G, Kaur CD, Saraf S. Development of novel herbal cosmetic cream with *Curcuma longa* extract loaded transfersomes for antiwrinkle effect. Afr J Pharm Pharmacol 2011;5:1054-62.
9. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes - Novel vesicular carriers for enhanced delivery: Characterization and skin penetration properties. J Control Release 2000;65:403-18.
10. Malakar J, Sen SO, Nayak AK, Sen KK. Formulation, optimization and evaluation of transferosomal gel for transdermal insulin delivery. Saudi Pharm J 2012;20:355-63.
11. Dave V, Kumar D, Lewis S, Paliwal S. Ethosome for enhanced transdermal drug delivery of aceclofenac. Int J Drug Deliv 2011;2:81-92.
12. Hamed R, Basil M, AlBaraghthi T, Sunoqrot S, Tarawneh O. Nanoemulsion-based gel formulation of diclofenac diethylamine: Design, optimization, rheological behavior and *in vitro* diffusion studies. Pharm Dev Technol 2016;21:980-9.
13. Lonni AA, Munhoz VM, Lopes GC, Longhini R, Borghi-Pangoni FB, Santos RS, *et al*. Development and characterization of multiple emulsions for controlled release of *Trichilia catigua* (Catuaba) extract. Pharm Dev Technol 201;21:933-42.
14. Patel MR, Patel RB, Parikh JR, Patel BG. Novel microemulsion-based gel formulation of tazarotene for therapy of acne. Pharm Dev Technol 2016;21:921-32.
15. Misal J, Dixit G, Gulkari V. Formulation and evaluation of herbal gel. Indian J Nat Prod Res 2012;3:501-5.
16. du Plessis J, Ramachandran C, Weiner N, Müller D. The influence of particle size of liposomes on the deposition of drug into skin. Int J Pharm 1994;103:277-82.
17. Verma DD, Verma S, Blume G, Fahr A. Particle size of liposomes influences dermal delivery of substances into skin. Int J Pharm 2003;258:141-51.
18. Elsayed MM, Abdallah OY, Naggar VF, Khalafallah NM. Lipid vesicles for skin delivery of drugs: Reviewing three decades of research. Int J Pharm 2007;332:1-16.
19. Chourasia MK, Kang L, Chan SY. Nanosized ethosomes bearing ketoprofen for improved transdermal delivery. Results Pharm Sci 2011;1:60-7.
20. Srivastava N, Singh K, Amrit K. Formulation and evaluation of seabuckthorn leaf extract loaded ethosomal gel. Asian J Pharm Clin Res 2015;8:316-20.
21. Paolino D, Celia C, Trapasso E, Cilurzo F, Fresta M. Paclitaxel-loaded ethosomes®: Potential treatment of squamous cell carcinoma, a malignant transformation of actinic keratoses. Eur J Pharm Biopharm 2012;81:102-12.

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