# DESIGN AND CHARACTERIZATION OF A TRANSDERMAL GEL CONTAINING ETHOSOME OF CARALLUMA FIMBRIATA PLANT EXTRACT

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***Abstract:*** *Caralluma fimbriata (Makadshenguli) belongs to the family of Asclepiadacea. The plant has anti-inflammatory, antibacterial, antimalarial, antioxidant, antipyretic, anthelmintic and antifungal activities. The stable gel formulation containing ethosomes loaded with Caralluma fimbriata extract was prepared and evaluated for topical drug delivery. The extract was screened for phytochemical investigation. It was quantified for total flavonoid content using instrumental techniques. Ethosomes were prepared using the cold method incorporating Caralluma fimbriata, in ethanol, propylene glycol and phospholipid. Caralluma fimbriata extract was incorporated in ethosomal preparation and the effect of phospholipid and ethanol concentration on the therapeutic action of Caralluma fimbriata were studied. The prepared ethosomes were evaluated by Design Expert Software and parameters such as Entrapment Efficiency (EE), Zeta Potential (ZP), Particle Size, and In-vitro drug release were studied. The prepared Ethosomal gel was evaluated by pH measurement, homogeneity, viscosity, spreadability, texture analysis, gelling time, drug content, and In-vitro drug release.*

**Keywords:** *Caralluma fimbriata,Cold method,Ethosomal gel, Anti-inflammatory, In vitro study.*

## INTRODUCTION

Caralluma fimbriata, sometimes called Carallumaadscendens, is a member of the family Asclepiadaceae. It is also known as Shindulamakadi, Ranshabar, Makadshenguli, and Kullimudayan in Western India. India is home to several Caralluma species. Varieties of Caralluma have been used traditionally to cure rheumatism, diabetes, leprosy, paralysis, and inflammation.Caralluma is a 20–30 cm, upright, branching plant. These plants also exhibit antimalarial, antitrypanosomally, anti-ulcer, antioxidant, antinociceptive, and antiproliferative properties.[1]

## MATERIAL AND METHOD

### Plant extract

Caralluma fimbriata extract (25%) procured from kshipra biotech private limited (Indore)
Quercetin was procured from sigma eldrichmumbai,india. Tetracycline disc 10 Mg was procured from himedia, india. All other substances were analytical grade.

### Preformulation studies of the selected drug

### Physical analysis

The extract was evaluated for physical appearance, taste and Oduor.

### Solubility determination

The amount of drug (around 1-2 mg) that was placed in the test tube with 5 ml of the solvent dissolve (water, ethanol, methanol and 7.4 pH buffer). It was forcefully shaken and kept for some period. The API was shown to be soluble in a number of solvents (at room temperature).[3]

**Melting point**

Melting point was calculated by taking a little quantity of powder attached intocapillary tube. The Capillary tube was placed in the melting point gauge (Chemline), which contained castor oil. Castor oil temperature is measured as it gradually expands, as the temperature at which the powder first begins to soften, and as the temperature at which the entire powder melts.[3]

**Determination of $\lambda_{max}$ and construction of calibration curve of Caralluma fimbriata**

Weighed at of 10 mg extract dissolved in 10 ml of water to form 1000 µg/ml, resultant solution. Further dilution made to obtained 100–600µg/ml solution. Then sample scanned in range of the 200-400 nm by using UV spectrophotometer. The wavelength at which minimum absorbance observed selected and analytical wavelength.Caralluma fimbriata absorbance versus wavelength was shown in a diagram showing the spectrum's climax point. Calibration curve performed in phosphate buffer (PH 7.4)and Quercetin in methanol was found to be linear in the concentration range of 2-10µg/ml used as standard.[3]

**Preliminary phytochemical screeningof extract**

The hydroalcoholic extract of Caralluma fimbriata was subjected to preliminary phytochemical test, like flavonoids, alkaloids, tannin's, saponin, phenols, and steroids, were found during the phytochemical screening.

**Thin LayerChromatography**

Thin Layer chromatography analysis was carried out for flavonoids in thesolvent system of ethyl acetate: formic acid: glacial acetic acid: water with a ratio of10:1:1:2.6 (v/v/v/v).

**FTIR analysis**

Fourier Transform Infrared Spectroscopy (FT-IR) was used to identify the active functional groups in the extract. Compatibility of drug was study. A pellet made from the dried materials and potassium bromide was compressed to 300 $kg/cm^2$ pressure. Using KBr discs, the spectra was captured in the 4000-600 $cm^{-1}$ range[5].

**Differential Scanning Calorimetry**

Thermal behavior of API was studied by using DSC Perkin Elemer at the heating rate of at 10 ° C. sample was accurately weighed in aluminium pan and sealed. Measurement was performed at heating range of 25° - 300° C under nitrogen atmosphere.[6]

**Quantitative analysis by uv spectrophotometry**

**Determination of to talflavonoid content (Colorimetric Method):**

The total flavonoid content of Caralluma fimbriata was evaluated using the aluminium chloride colorimetric method. This approach was modified from that used by Afify et al. (2012) but was based on their methodology. 0.5 ml of the sample (1 mg/ml) was combined with 1 ml of potassium acetate (1 M), 1 ml of aluminium chloride (10%), and 2.5 ml of distilled water. In order to create the calibration curve, quercetin was utilized. Using a UV-spectrophotometer, the mixture's absorbance at 415 nm was determined. In terms of quercetin equivalent, the total flavonoid concentration was reported as mg QE/g of the sample. Three times through all the analyses, the mean absorbance value was acquired[7].

**Experimental Procedure**

The concentration was 1 mg/ml or 1000 µg/ml was prepared after 50 mg of quercetin were dissolved in 50 ml of methanol.In each test tube, 0.5 ml of stock solution was added.Then 1ml of 10% Alcl3 and

Potassium acetate (1 ml) added into the test tube followed by addition of 2.5 ml of distilled water.
To complete the reaction, the test tube was kept at room temperature for 30 minutes.
Utilizing a spectrophotometer, the concentration of the solution was determined at 415 nm.
The following formula was used to determine the total amount of flavonoid component in plant extract comparable to quercetin:[13]
[C = (c×v`)/ M]
were,
C = Total flavonoid content mg/g plant extract, in quercetin Equivalent (QE)
c = Concentration of quercetin from the calibration curve mg/ml,
v = volume of extract in ml
M = weight of pure plant in mg

**Method of preparation of Ethosomes**
**Cold method**
Carallumafimbriataetho some were produced using the cold technique. Extract and soya lecithin were dissolved in ethanol with vigorous shaking in a small round bottom flask. To stop ethanol from evaporating, a cap was placed over the flask's spherical bottom. Then, to the ethanolic mixture, propylene glycol is added. The ethanolic mixture was then warmed to 30°C in a water bath. In a separate beaker, distilled water was heated to 30°C before being carefully introduced and stirred into the middle of the ethanolic mixture for 5 minutes. The system was maintained at 30°C while the mixing proceeded for an additional 15 minutes. Using probe sonication for three cycles of five minutes each, followed by a five-minute break, the ethosomal formulation's vesicle size was decreased to the nano-size range. The combination was kept chilled [8,9]. The batches B1–B9 were prepared as follows Table no. 1.

**Table 1: Trial preliminary batches**

| Batches | Drug (mg) | Soya lecithin (mg) | Ethanol (ml) | Propylene glycol (ml) | Distilled water (ml) |
|---|---|---|---|---|---|
| B1 | 30 | 30 | 3 | 2 | 5 |
| B2 | 30 | 30 | 3.5 | 1.5 | 5 |
| B3 | 30 | 30 | 4 | 1 | 5 |
| B4 | 30 | 65 | 3 | 2 | 5 |
| B5 | 30 | 65 | 3.5 | 1.5 | 5 |
| B6 | 30 | 65 | 4 | 1 | 5 |
| B7 | 30 | 100 | 3 | 2 | 5 |
| B8 | 30 | 100 | 3.5 | 1.5 | 5 |
| B9 | 30 | 100 | 4 | 1 | 5 |

**The prepared ethosomes were evaluated for following parameter:**
**Determination of particle size**
Lecithin and ethanol influence the vesicle size. The size of the vesicle was shown torise as the concentration of soya lecithin increased. This could be due to the lipid forming thevesicle wall, resulting in increased wall thickness and, eventually, vesicle size. The particlesize of ethosome ranges from 10 nanometers (nm) to a few microns (μ). The particle size less than300 nmshowseffectiveskin penetration[10].

**Polydispersity index**
Polydispersity, also known as the heterogeneity index, is the degree of non-uniformity in a particle size distribution. Values lower than 0.05 are often only seen with severely monodisperse standards due to the dimensional lessness of this measure's scale. PDI values over 0.7 suggest wide particle size dispersion in the sample. Following is the formula for calculating PDI:

$X_{50}$-$X_{10}$÷$X_{90}$

**Determination of entrapment efficiency (%EE)**

Entrapment efficiency was calculated by taken 2 ml of the formulation and centrifuged at 7000 rpm in an ultracentrifuge for 20 minutes to determine the entrapment efficiency. Supernatant was separated from the sediment. Water in the amount of 10 ml was added to the sediment. A UV spectrophotometer calibrated at 281.8 nm was used to calculate the volume of the extract. The effectiveness of encapsulation or entrapment was calculated in relation to the initial drug concentration added using the following equation:
EE% =(Totaldrug–Free Drug ÷Total drug)×100

**Table 2: Trial Preliminary Batches**

| **Factor 1** | **Factor 2** | **Factor 3** | **Response 1** | **Response 2** | **Response 3** |
|---|---|---|---|---|---|
| A: Soya Lecithin | Ethanol | Propylene Glycol | Entrapment Efficiency | Particle Size | Zeta potential |
| mg | ml | ml | % | nm | mV |
| 40 | 4 | 0.5 | 81 | 160 | -36.5 |
| 40 | 5 | 1 | 80 | 1043 | -2.4 |
| 30 | 4 | 1 | 79 | 178 | -36.5 |
| 20 | 5 | 1 | 86 | 412 | -31.7 |
| 30 | 4 | 1 | 79 | 178 | -36.5 |
| 30 | 3 | 0.5 | 81 | 164 | -36.5 |
| 30 | 5 | 1.5 | 79 | 275 | -35.2 |
| 30 | 4 | 1 | 79 | 178 | -36.5 |
| 30 | 3 | 1.5 | 78 | 134 | -46.5 |
| 40 | 3 | 1 | 76 | 194 | -33.4 |
| 30 | 4 | 1 | 79 | 178 | -36.5 |
| 30 | 4 | 1 | 79 | 178 | -36.5 |
| 20 | 4 | 0.5 | 82 | 158 | -40.3 |
| 20 | 3 | 1 | 78 | 120 | -45.5 |
| 20 | 4 | 1.5 | 81 | 202 | -38.6 |
| 40 | 4 | 1.5 | 76 | 180 | -32.6 |
| 30 | 5 | 0.5 | 84 | 266 | -35.5 |

**Determination of zetapotential**

Zeta potential is an important and useful indicator of particle surface charge, which can be used to predict and control the stability. Optimized ethosome zeta potential was measured using zeta sizer nano ZS90. Improved permeability occurs through negative zeta potential, increase with increase ethanol concentration, while soya lecithin has an inverse relationship.

**Determination of drug content**

Drug content was calculated by weighing 1 ml of the ethosome complex and dissolving it in 10 ml of water. Following the appropriate dilution, the drug concentration was ascertained by measuring the absorbance using a UV spectrophotometer at 281.8 nm.[11]

**Selection of optimized batch of ethosome**

Optimized batch selected on established parameters suchas particle size, entrapment efficiency, high drug content and effective in-vitro drug release, an optimum formulation was selected.

**Experimental design**

By using box-behnkendesign from design expert software, the experimental design was carried out. Three components were statistically analyzed in this design, each at three levels, and preliminary tests were carried out. The dependent variables were entrapment efficiency in % (Y1), particle size in nm (Y2), and zeta potential in mV (Y3), while the independent variables were the phospholipid in mg (X1), ethanol in ml (X2), and propylene glycol in ml (X3). Using Design Expert software, a number of models,including 2FI, Cubic, and Quadratic, were fitted to the data for a single answer. Analysis of variance was used to statistically examine the collected data (ANOVA).

**Table 3: Optimized batch**

| **Run** | **Drug** | **Factor 1** | **Factor 2** | **Factor 3** | **Response 1** | **Response 2** | **Response 3** | **-** |
|---|---|---|---|---|---|---|---|---|
| | H-CFE | Soya lecithin | Ethanol | Propylene Glycol | Entrapment Efficiency | Particle Size | Zeta potential | Polydispersity index |
| | mg | mg | ml | ml | % | nm | mV | - |
| 18 | 30 | 20 | 4.6 | 0.5 | 83 | 156 | -2.4 | 0.09026 |

**Contour plots and surface responsecurves**

Diagrammatic depictions of the response values include contour plots and surface response curve which aid in illuminating how dependent and independent variables are related. To investigate the effects of phospholipid, propylene glycol, and ethanol on the dependent variables, namely entrapment efficiency (percent EE), particle size (nm), and zeta potential(mV), the data were submitted to a 3-D response surface approach and contour plot.[12]

**Transmission electron microscopy**

Surface morphology was determined by TEM. For TEM a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aqueous solution of phosphotungustic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron at magnifications of 27800x, 4640x, and 60,000x, digital pictures of the ethosome of Caralluma fimbriata were captured.[13]

**Preparation of ethosomal gel**

Ethosomes were prepared by mixing 50 ml of distilled water was mixed with 1 g of Carbopol 934. with constant swirling, the calculated quantity of methyl paraben and propyl paraben were dissolved in 5 ml of distilled water by boiling on a water bath. Propylene glycol 400 was then added when the solution and cooled. The above combination was then combined with the additional ethosomal dispersion that was necessary, and the remaining distilled water was added upto 100 ml. After thoroughly combining all of the components with constant stirring to create the Carbopol 934 gel, triethanolamine was added drop by drop to the mixture to adjust the pH to the desired range (6.8–7) and create the gel's desired consistency. The production of the control sample of Caralluma fimbriata extract used the same procedure.

**Preparation of H-CFE gel**

The direct dispersion method was used to make the 100 g gel. Following the dissolution of propyl paraben and methyl paraben in water at 80°C, Carbopol 934 was precisely weighed and then dissolved in wat at 40°C with continuous stirring for 30 min. A precisely measured amount of the extract dissolved in a small amount of water before being continuously stirred into the Carbopol solution All of the components with constant stirring to create the Carbopol 934 gel. To the gel triethanolamine was added drop by drop to the mixture to adjust the skin's pH to the desired range (6.8–7) and create the gel's desired consistency.

**Characterization of ethosomal Gel**

**Physical examination and homogeneity**
The prepared ethosomes and ethosomal gel formulations were inspected visually for their color intensity difference. All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container.They were also tested for their appearance and presence of any aggregates.[14]

**Determination of spreadability**
Spreadability was determined by apparatus which was suitably modified in the laboratory and used for the study. The spread ability was evaluated using specially designed wooden block and glass slide equipment. The upper slide (movable slide) was totally separated from the fixed slide after a weight was placed to the pan. This time was observed.[15]
Then, spread ability was determined using the formula
$S = M*L/T$
were
S- stands for spread ability and M for weight of the tide to upper slide.
L - The glass slide's length.
T - The length of time it took to fully isolate each slide from the others.

**pH measurement**
The pH was checked using a pH meter. The normal pH range should be in the range of 4-6 [16].

**Viscosity**
Thermostatically controlled Brookfield viscometer (Brookfield LVDV III, Brookfield Engineering Laboratories, Middleboro, MA) equipped with a CP-52 spindle at 30, 50, and 100 rpm, respectively, the viscosity of the generated formulations was determined. [17]

**Texture analysis**
Analysis of the texture profile is a useful way to identify the characteristics of polymeric systems. A Texture Analyzer TA-XT2 (The experiments were conducted at Digital Scientific Equipment, RK Puram,). The gels were inserted into a normal beaker underneath the probe to conduct the experiment. The sample is subsequently submerged in an analytical probe in this process. The "gelling strength test" or compression mode of the Texture Analyzer was selected, and the test speed was set to 1.0 mm/s. A trigger force of 5 g and an acquisition rate of 50 points per second were used. Each sample was taken using a 7.6 cm diameter aluminium probe. The research was conducted at room temperature.

**Gelling time**
A vial of ethosome gel was placed in an incubator and kept at a temperature of 37°c to measure the gelling capacity. The gel formation was seen visually, and the times needed for gelation and sol to gel formation were recorded.[18]

**Skin permeation study**
The drug release studies were carried out using modified franz diffusion cell. Samples were withdrawn at°maintained at 37±0.2 different time interval and compensated with same amount of fresh dissolution medium. Volume of sample withdrawn was made up to 10ml by PBS (pH 6.8). Cellophane membrane was positioned between the donor compartment and the receptor compartment, which were clamped together. The 8-hour experiment was conducted.The sample was removed at predefined intervals of 30 min. Quercetin content was instantly determined by UV spectrophotometer at 256 nm.[19]

**Stability study**
The Caralluma fimbriata extract ethosome was exposed to stability conditions. The ideal formulation was stored for a month at three different temperatures and relative humidity levels: 5°C ± 3°C, 30°C ± 2°C/65% RH, and 40°C ± 2°C/75% RH. [20,21]

**Drug content analysis**
A specified quantity (10 mg) of the prepared extract ethosomal gel was dissolved in 10 mlof water.The volumetric flask wassonicatedfor 20 mins toallowthe extracttodissolve completely. The solution was filtered, and drug content was determined using theUV spectrophotometricmethod at281.8 nmusing water asablank medium.

## RESULT AND DISCUSSION

Preformulation studies are essential protocols for improvement of safety, efficacy and stability of dosage form as well.

**Melting point Determination**
The melting point of the drug was determined by capillary method where it is introduced into digital melting point apparatus the temperature range at which drug melts was found to be 132-135°C.

**Qualitative phytochemical screening**
The hydroalcoholic extract of Caralluma fimbriata was subjected to preliminary phytochemical test, like flavonoids, alkaloids, tannin's, saponin, phenols, and steroids, were found during the phytochemical screening.

**Table 4: Qualitative Phytochemical Analysis**

| Sr.no. | Phytochemicals | Test | Results |
|---|---|---|---|
| 1. | Alkaloids | a.Wagner's Test | +++ |
| | | b.Dragendorff's Test | +++ |
| 2. | Tannin's | a.LeadAcetate | - |
| | | b.5 % $Fecl_3$ | - |
| 3. | Flavonoids | ShinodaTest | ++ |
| 4. | Steroid | SalkowskiTest | ++ |
| 5. | Glycosides | Keller-KillaniTest | ++ |
| 6. | Saponin | Foam Test | +++ |
| 7. | Phenols | Testfor Phenol | - |
| 8. | Coumarins | TestforCoumarins | ++ |

Note: - +Low, ++ Moderate, +++ High, --- Absent.

**Thin layer chromatography**
Thin layer chromatography was done for hydroalcoholic extract of Caralluma fimbriata using quercetin as standard. The TLC plates were exposed to iodine chamber and observed under UV light. The separated compounds' Rf values were measured and recorded in Table No. 5.

**Table 5: $R_f$ values of TLC study**

| Sr.No. | Component | $R_f$value |
|---|---|---|
| 1 | Standard(Quercetin) | 0.9 |
| 2 | Extract | 0.83 |

**Quantitative analysis byUV spectrophotometry**
The Regression value was found to be value 0.9968 at concentration 15-75µg/ml
The total flavonoid content of hydroalcoholic extract Caralluma fimbriata was calculated and found to be

43µg/ml.
= 0.0067x + 0.0062
(Y - 0.0062) ÷ 0.0067= X
X = (0.2943 -0.0062) ÷ 0.0067
X = 43 µg/ml

**Compatibility studies**
FTIR analysis:
By using FT-IR Spectrophotometer, the functional groups found in the hydroalcoholic extract were identified. The sample's spectral representation is listed in Table No. 6 and shown visually in Fig. No. 1.

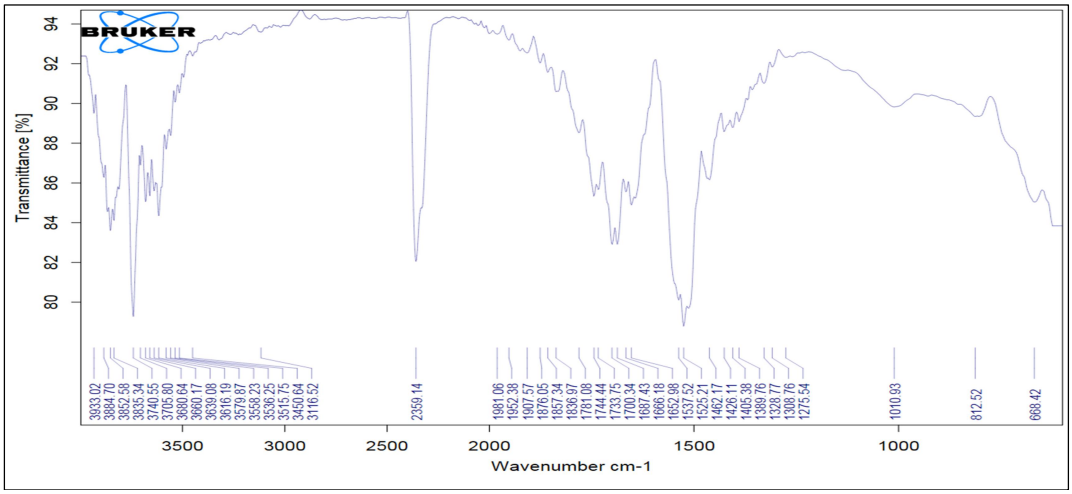



**Figure 1: FTIR spectrum of a hydroalcoholic extract of Caralluma fimbriata**

Quercetin's FTIR spectrum showed the distinctive signals due to O-H stretching at 3410 cm $^{-1}$, 1610 cm$^{-1}$ (C=O stretching in the fatty acid ester), 1444 cm$^{-1}$ (C=C stretch), 1285 cm$^{-1}$ and 1103 cm$^{-1}$, other signals were also seen. Different peaks were visible in the spectrum of the hydroalcoholic extract of Caralluma fimbriata at 3450 cm$^{-1}$ (O-H alcohol), 1537 cm$^{-1}$ (C=O Stretch), 1525 cm$^{-1}$ (C=C Stretch), 1275 cm$^{-1}$ and 1010 cm$^{-1}$ (C-O Stretch).

**Table 6: Spectral interpretation of FTIR Spectra of hydroalcoholic extract of Caralluma fimbriata and Quercetin**

| Sr. No. | Quercetin | Extract | Functional group | Possible secondary metabolites |
|---|---|---|---|---|
| 1 | 3410 cm$^{-1}$ | 3450 cm$^{-1}$ | O-H (alcohol) | Alkaloids, flavonoids, tannins, saponins, polyphenols, carbohydrates, steroids, terpenoids, carboxylic acid containing phytochemicals, etc. |
| 2 | 1610 cm$^{-1}$ | 1537 cm$^{-1}$ | C=O (stretch) | |
| 3 | 1444 cm$^{-1}$ | 1525 cm$^{-1}$ | C=C (stretch) | |
| 4 | 1285 cm$^{-1}$<br>1103 cm$^{-1}$ | 1275 cm$^{-1}$<br>1010 cm$^{-1}$ | C-O (stretch) | |

**FTIR**

The FTIR peak matching method was used to assess the compatibility between the hydroalcoholic extract and soya lecithin. In contrast to the hydro-alcoholic extract of Caralluma fimbriata, phytoconstituents showed a shift of the OH group to a lower frequency in the FTIR spectrum (3740.55 $cm^{-1}$ to 3673.66 $cm^{-1}$), indicating a strong hydrogen bond between the hydroxyl group of phosphatidylcholines and the phytoconstituents.

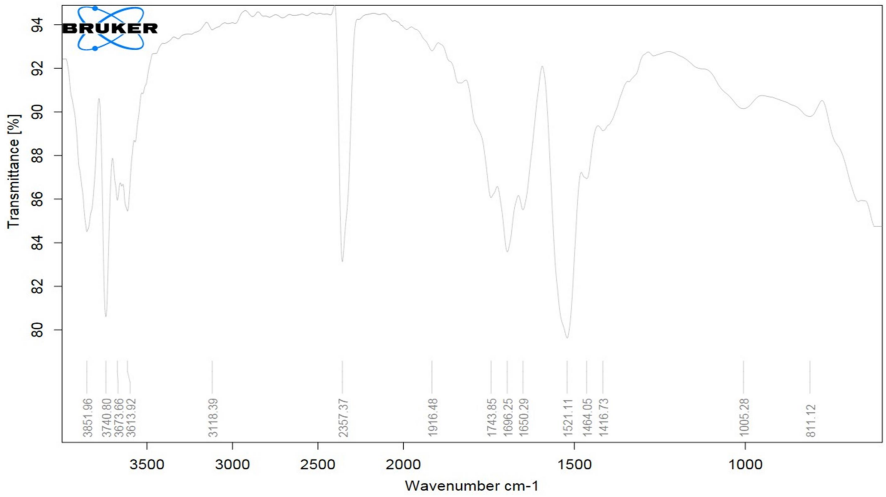
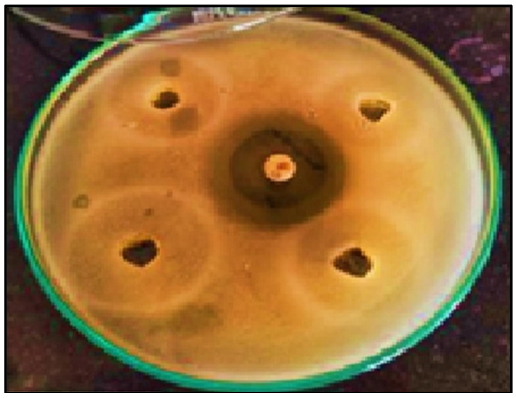



**Figure 2: FTIR spectrum of soya mixture (Extract and soya lecithin)**

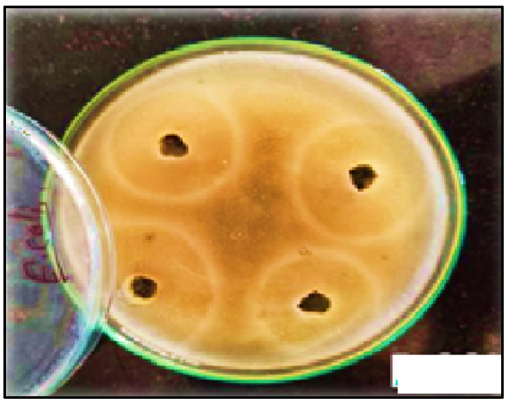
**In vitro antimicrobial activity of hydroalcoholic extract and ethosome**

**Table 7: Antibacterial assay parameters**

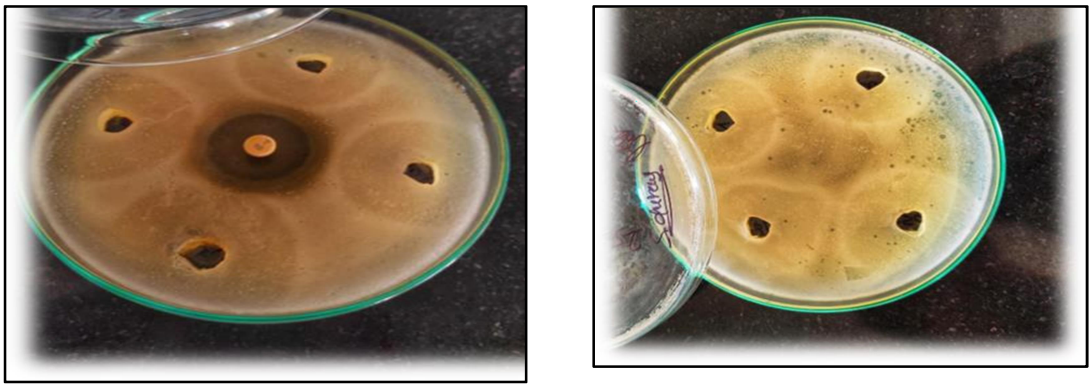
| **Sr. No.** | **Method** | **Well disc diffusion method** |
|---|---|---|
| 1 | Standard | Tetracycline disc |
| 2 | Solvent | Water |
| 3 | Bacterial Strain (Gram +ve) | S. Aureus |
| 4 | Bacterial Strain (Gram – ve) | E. Coli |



E coli



E coli



S. aureus S. aureus

**Figure 3: Antimicrobial Study of Extract (100-1000ppm)**

**Evaluation parameter for trial batches**

The formulated ethosome of Caralluma fimbriataextract were further evaluated for particle size, polydispersity index, drug entrapment efficiency, drug content, and in vitro drug release.

**Particle size**

The mean particle size for B1- B17 batches were found to be in the range of 120-1043 nm. As seen in Table 2. The final batch of formulated ethosome of Carallumafimbriata extract had particle size found to be 156nm as seen in Table 3.

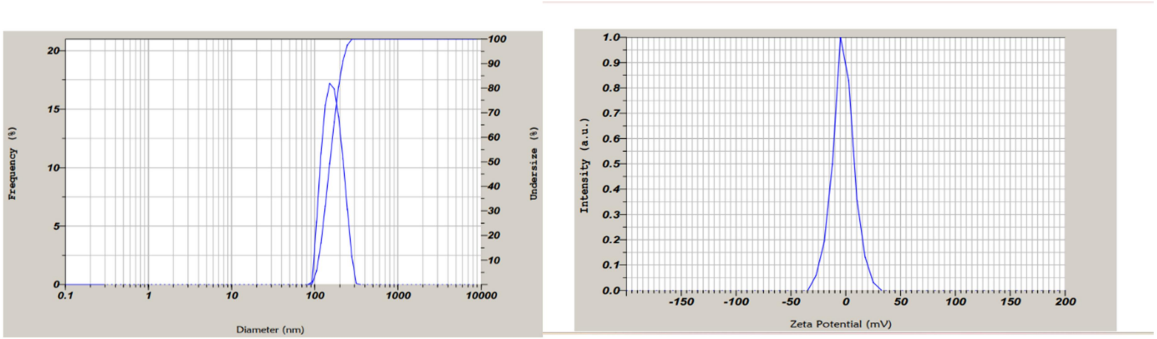



**Figure 4: Particle Size and Zeta Potential**

**Polydispersity index**

The formulated ethosome of Caralluma fimbriata extract had an average PDI value ≤ 0.3 indicating a homogenous distribution of ethosome as seen in Table 3.

**Zeta potential**

The zeta potential value is shown in Table 3. A zeta potential value of the optimized formulation is negative, indicating a stable formulation.

**Drug entrapment efficiency**

Entrapment efficiency was found to be between 76-86%. It is defined as the fraction of the drug absorbed into formulations relative to the total amount of drug used. Estimation of entrapment efficiency is a vital factor with respect to ethosome. As summarized in Table 2 formulations B1 and B18 showed maximum entrapment efficiency. From the result of entrapment efficiency, B18 batch was selected from the resul to fentrapment efficiency.

**In vitro drug release**

In vitro drug release studies of Caralluma fimbriata extract were performed in pH 7.4 PBS. For 8 hr. The release pattern in Figure shows a release pattern, where almost 65% of drug was released in the initial phase. Between the 1st and 2nd hr, the release is seen constant from 65 to 89%. Later on, within the last hour i.e., 3rd and 4th, we were able to see the drastic major release of drugs This accounts for the burst phase where almost all the unentrapped drug would be released. During the final phase, a comparatively sustained pattern of release of drug from ethosomes was observed for about 8 h.

**Optimized batch**

Depending on the particle size, entrapment efficiency, and zeta potential, formulation B18 was chosen as the optimized batch and was selected for further study due to its minimum particle size of 156 nm, high % entrapment efficiency of 83% and zeta potential -2.4 mV.

**Characterization of optimized batch of ethosome**

The characterization of optimized batch B18 was done by FT-IR Spectroscopy, differential scanning colorimetry and transmission electron microscopy.

**FT-IR Spectroscopy**

The band of the choline N-(CH3) group in phosphatidylcholine spectra is moved to a higher frequency in ethosome spectra (1005.28-1043.59 $cm^{-1}$) with a reduction in intensity, showing interaction between phosphatidylcholine and extract component at level of choline moiety.

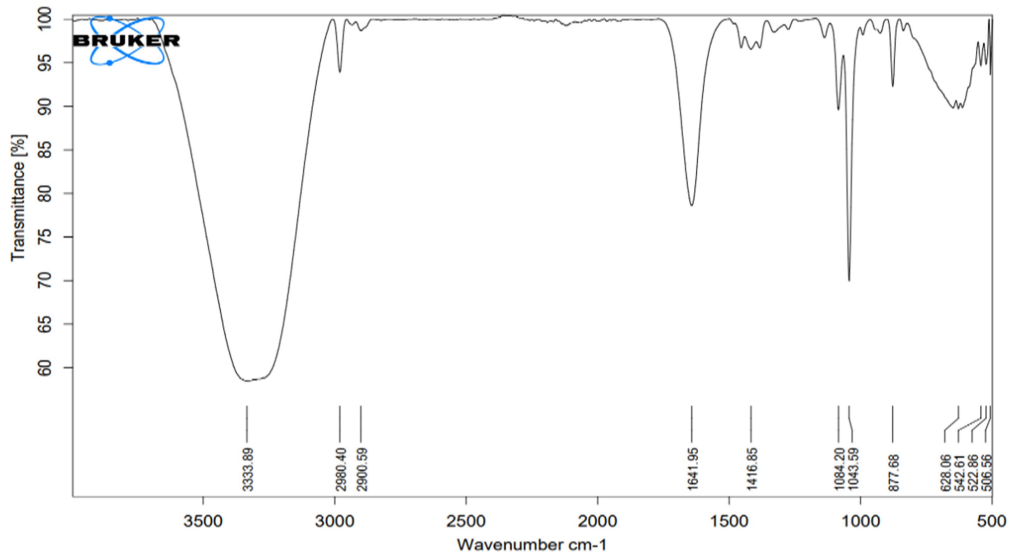



**Figure 5: FTIR spectrum of ethosome of Caralluma fimbriata**

**Transmission electron microscopy**

Morphological characteristics of ethosome were evaluated using transmission electron microscopy. Samples were diluted with water and sonicated for 10 min. A drop of ethosome was fixed onto a carbon glazed grid, left to form a thin film and the ethosome images were taken using TEM. The ethosome were scanned in the range of 50- 300 nm. The TEM view of optimized batch of Caralluma fimbriata hydroalcoholic extract showed sphere shaped vesicles.

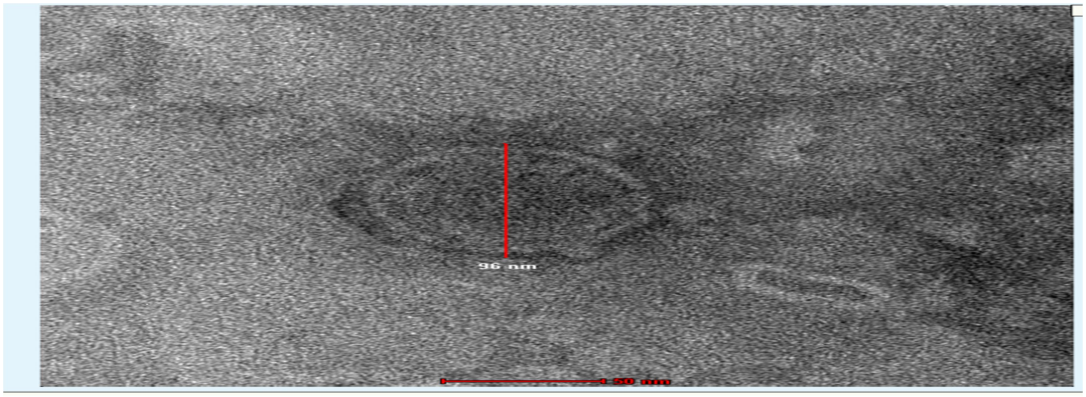



**Figure 7: TEM view at 50 nm**

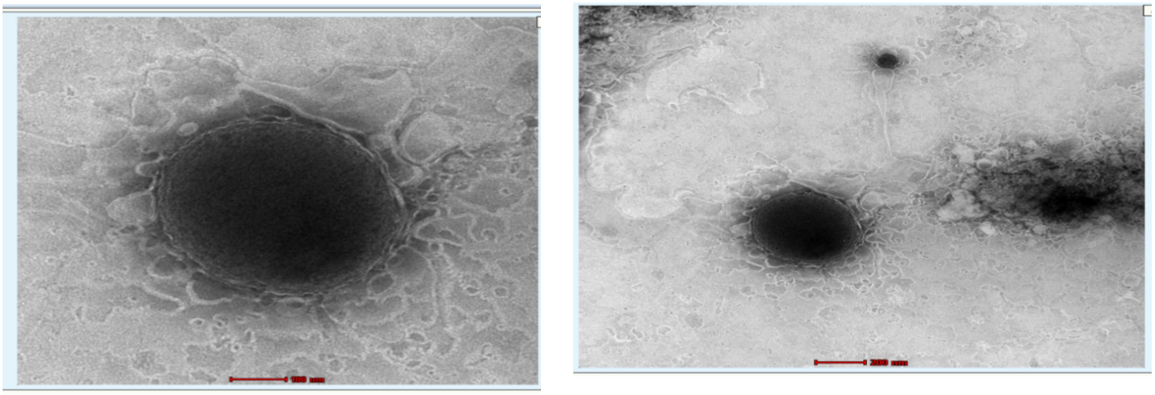
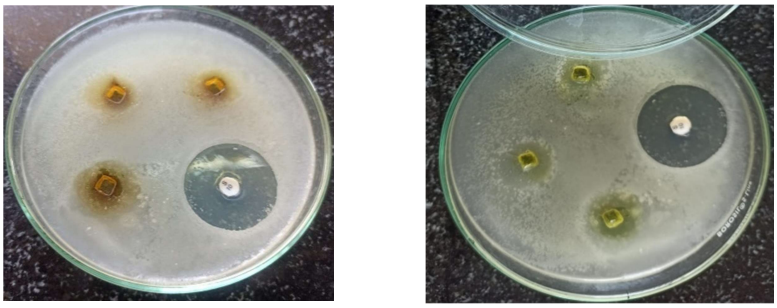


**Figure 8: TEM view at 100 nm and 300nm**

**In- vitro antimicrobial activity of ethosome of Caralluma fimbriata extract:**
Gram-positive bacteria like Staphylococcus aureus and gram-negative bacteria like Escherichia coli were tested for antibacterial activity against the ethosome of the Caralluma fimbriata.
The ethosomal gel of Caralluma fimbriata showed enhanced zone of inhibition as compared to hydroalcoholic extract of Caralluma fimbriata. Because of its smaller particle size and improved penetration of bacterial cell walls, ethosome antibacterial action has been improved.



*a. Staphylococcus aureus*

b. *Escherichia*

**Figure 9: Antimicrobial study of ethosome (50-200 ppm)**

**Table 8: Antmicrobial activity of ethosome of Caralluma fimbriata extract against pathogen.**

| Sr. No. | Concentration (ppm) | S. aureas(mm) | E. coli (mm) |
|---|---|---|---|
| 1 | Standard (Tetracycline 10mcg) | 23 | 24 |
| 2 | 50 | 14 | 12 |
| 3 | 100 | 16 | 14 |
| 4 | 150 | 18 | 16 |
| 5 | 200 | 24 | 22 |

**Preparation of ethosomalgel**

The topical gel was prepared using Carbopol at 1% concentration with B18 Ethosomalgel.Further prepared gel was subjected to physicochemical properties like, pH measurement, drug content &in-vitrodrug release studies.

**Prepared ethosomal gel was subjected to following parameter**

**Homogeneity**

The appearance was checked visually. The ethosomal gel was found to be Faint brown.

**pHmeasurement**

The pH of the created formulations was determined using an elico india systronics digital pH meter. ThepH of thegelwas found to be 5.7, which is suitablefor human skin.

**Viscosity**

**Table 9: Viscosity of H-CFE and ethosomal gel**

| Sr. no | Viscosity | |
|---|---|---|
| | H-CFE Gel | Ethosomal Gel |
| 1 | 2287cpc | 3791 cpc |
| 2 | 2190 cpc | 3695 cpc |
| 3 | 2040 cpc | 3540 cpc |
| mean | 2172.3 cpc | 3675.3 cpc |

Mean viscosity for batch B18 at H-CFE and ethosomal gel were calculated and at room temperature mean viscosity was found to be 3695.3cps which was adequate for ease of Spreadability.

**Texture analysis**

**Table 10: Gel samples were evaluated using texture analyzer for following properties**

| Formulation | Firmness (gm) | Tackiness (gm) | Work of adhesion |
|---|---|---|---|
| H-CFE gel | 343.924 | 495.472 | 1.440645 |
| Ethosome loaded gel | 417.245 | 495.1085 | 1.186643 |

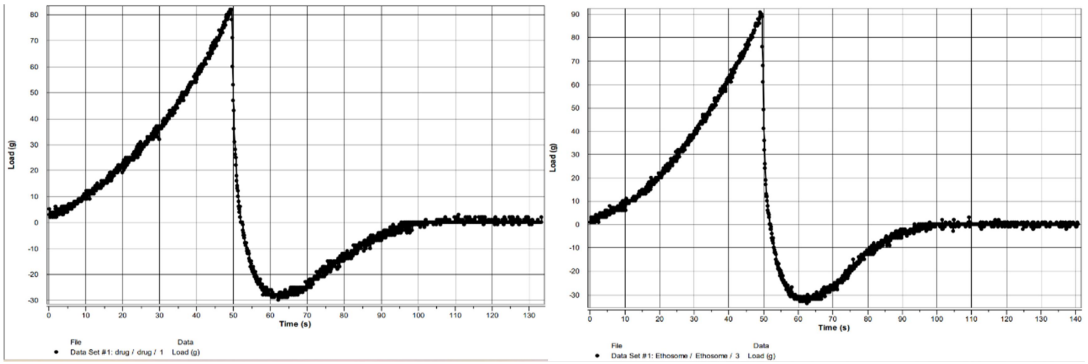



**Figure 10: Texture analysis of H-CFE and ethosomal gel**

**Drug content analysis**
The drug content of ethosomal gel was found to be86 %

**Prepared ethosomal gel was subjected to following test**
The ethosomal gel was prepared and evaluated for in-vitro drug release

**In-vitro drug release**
The % drug release was shown below in Table No. 11 and Fig. No.11indicates an 95 % drug release from ethosomalgel.

**Table 11: % Drug release of ethosomalgel**

| Sr. No | Time(hr) | H-CFE | Ethosomal Gel |
|---|---|---|---|
| 1 | 0.5 | 65 | 53 |
| 2 | 1 | 84 | 68 |
| 3 | 2 | 89 | 71 |
| 4 | 3 | 94 | 70 |
| 5 | 4 | 97 | 79 |
| 6 | 5 | -, | 82 |
| 7 | 6 | - | 85 |
| 8 | 7 | - | 89 |
| 9 | 8 | - | 95 |

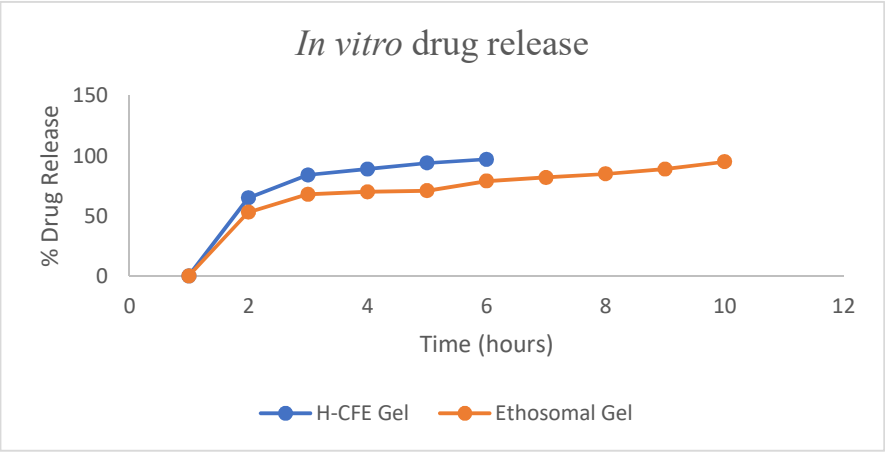



**Figure 11: % Drug release of ethosomal gel**

**Stability studies**

The optimized formulation was sealed and stored at 5°C ± 3°C, 30°C ± 2°C/ 65 % RH and 40°C ± 2°C/75 % RH for a time period of 30 Days. From the results it was evident that in the formulation no significant change was observed in entrapment efficiency with stored in refrigerator and room temperature. Thus, we conclude that the drug does not undergo degradation on storage.

**Table 12: Stability result of entrapment efficiency of ethosomal gel**

| Sr. No. | Stability Conditions | Time (days) | Entrapment Efficiency of Ethosomal Gel |
|---|---|---|---|
| 1 | 5 ± 3°C, 30°C ± 2°C/ 65 % RH, 40°C ± 2°C/75 % RH | 0 | 94.4 % |
| | | 7 | 95.2 % |
| | | 14 | 96.6 % |
| | | 21 | 96.5 % |
| | | 30 | 96.2 % |

## CONCLUSION

In the current study, Caralluma fimbriata extract of phospholipid complex were prepared using a cold method and evaluated using various physiochemical parameters. The investigation of physiochemical parameters revealed that hydroalcoholic extract of Caralluma fimbriata formed complexes with phosphatidylcholine that had higher bioavailability. The complex's formation was confirmed by IR, DSC, and TEM studies. The complex's dissolution profile was found to be improved. As a result, the phospholipid complex of hydroalcoholic extract of Caralluma fimbriatacan therefore be concluded to have the potential to enhance bioavailability.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## SUMMARY

The plant of Caralluma fimbriata extract belonging to the family Asclepiadaceaewere used and investigated for design, formulation, evaluation of ethosome, and antimicrobial activity for hydroalcoholic extracts.Eighteen formulations were prepared from which B18 showed significant results for anti-inflammatory activity.

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