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FORMULATION DEVELOPMENT AND CHARACTERIZATION OF ETHOSOME OF *MILLINGTONIA HORTENSIS* LEAVES EXTRACT

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ABSTRACT

The primary objective of this study was to develop and evaluate ethosomal formulations incorporating ***Millingtonia hortensis*** leaf extract. Ethosomes were prepared using the cold method, with each formulation containing 30 mg of drug and varying proportions of ethanol, propylene glycol, and soy lecithin in 5 mL of distilled water.

The ethosomes were characterized using Fourier Transform Infrared Spectroscopy (FTIR), zeta potential analysis, particle size determination, entrapment efficiency evaluation, and drug release studies. An ethosomal gel was subsequently formulated using the dispersion technique. The gel was assessed for drug content, in vitro drug release, and pH.

The optimized B9 formulation demonstrated a particle size of 226.54 nm, a zeta potential of -24.60 mV, and a pH range of 4–6. This optimized formulation exhibited enhanced penetration properties, indicating its potential as a promising candidate for improving bioavailability.

Keywords: *Millingtonia hortensis*, Ethosome, Cold method, Evaluation, Penetration, Soy lecithin

INTRODUCTION

Millingtonia hortensis *Linn*., a widely recognized medicinal plant, is extensively utilized across Southern China, Thailand, Myanmar, and India. Its diverse pharmacological properties have made it a focal point of interest in traditional medicine, where it is valued for its antibiotic potential, lung-tonic effects, and efficacy in managing asthma. The leaves of **M. hortensis* * are traditionally employed for their therapeutic benefits, including cholagogue, tonic, antipyretic, and antiasthmatic activities, underscoring its significance in ethnomedicinal practices **[1]**. The stratum corneum, composed of lipid bilayers, serves as a major barrier to drug permeation into the skin. To overcome this challenge, novel formulations such as ethosomes have been developed. According to Touitou et al. (2000), ethosomes primarily consist of phospholipids and ethanol. Ethanol, acting as a skin permeation enhancer, facilitates the transdermal delivery of therapeutic agents. Many previous studies have demonstrated the efficacy of ethosomes in enhancing drug penetration through the skin, highlighting their potential in dermatological and transdermal applications **[2]**.

Ethosomes offer a non-invasive approach for drug delivery, facilitating the penetration of therapeutic agents into the systemic circulation or deeper epidermal layers. These flexible, deformable vesicles are specifically designed to enhance the distribution of active pharmaceutical ingredients. Additionally, ethosomes are capable of delivering a wide range of drugs, including hydrophilic, lipophilic, and amphiphilic compounds, making them versatile carriers in drug delivery systems **[3]**. Ethosomes represent a modified form of the well-established drug delivery carrier, liposomes. These lipid-based vesicles primarily consist of water, phospholipids, and a high concentration of alcohol, such as ethanol and isopropyl alcohol, which contribute to their unique properties and enhanced drug delivery capabilities **[4-5]**.

The objective of this innovative approach was to enhance the transdermal penetration of active substances. This study specifically investigates the potential of ***Millingtonia hortensis*** in this context. Traditional transdermal formulations often face challenges in achieving optimal bioavailability of herbal constituents due to the skin barrier. However, ethosomal drug

delivery systems have demonstrated significant potential in overcoming these limitations. By utilizing ***Millingtonia hortensis*** leaf extract, this study provides a foundation for future research focusing on the transdermal application of other unexplored natural compounds. The formulation of ethosomes in this study is guided by existing literature on the therapeutic properties of ***M. hortensis***.

MATERIAL AND METHOD:

The leaves of **Millingtonia hortensis** were collected in November from Solapur, Maharashtra, and authenticated by the Botanical Survey of India, Koregaon Road, Pune (Specimen No. PNRMH1). Propylene glycol, ethanol, and soy lecithin were procured from New Neeta Chemicals, Pune, for use in the study.

Experimental work:

Extraction of *Millingtonia hortensis* L.

The leaves of **Millingtonia hortensis** were air-dried and subsequently extracted using 50% ethanol. Soxhlet extraction was carried out at 75°C, with 20 grams of powdered plant material used per batch. The resulting extract was concentrated to one-third of its initial volume using a rotary evaporator under reduced pressure. The final greenish-black extract was stored at 8°C for further use **[6]**.

Experimental design:

The experimental design was developed using Design Expert software. The Central Composite Design (CCD), a response surface methodology, was employed to optimize the formulation. In this study, entrapment efficiency (Y1) served as the dependent variable, while the proportions of phospholipid (X1) and ethanol (X2) were the independent variables.

Table 1: Ethosomes formulation composition

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

Preparation of *Millingtonia hortensis* Extract Loaded Ethosomes:

The cold method was employed to prepare the ***Millingtonia hortensis*** ethosomes. Soy lecithin and the plant extract were dissolved in ethanol by vigorously shaking a small round-bottom flask. In a separate beaker, distilled water was heated to 30°C and then gradually added to the ethanolic mixture while stirring for five minutes. The system was maintained at 30°C and stirred for an additional 15 minutes. To reduce the vesicle

size to the nanoscale, the formulation underwent three cycles of sonication, each lasting five minutes, with a five-minute interval between cycles.

Evaluation parameter:

Organoleptic Evaluation:

The organoleptic properties of the formulation, including color and odor, were assessed visually.

Zeta Potential and Particle Size:

Zeta potential, which measures the magnitude of electrostatic repulsion or attraction between particles, was determined using a zeta potential analyzer. The sample was diluted to 1:100 and placed in a cuvette for analysis.

Determination of Entrapment Efficiency (EE):

Entrapment efficiency was determined by centrifuging three Eppendorf tubes (n = 3) containing aliquots from each formulation at 7000 rpm for 20 minutes at 4°C. The supernatant was separated, filtered, and the amount of free drug was quantified using UV spectrophotometry at 256 nm. The entrapment efficiency was calculated using the formula:

EE% = (Total Drug- Free Drug / Total Drug) *100

Polydispersity Index (PDI):

The particle size distribution was assessed using the polydispersity index (PDI). A PDI value of less than 0.4 indicates a homogeneous, monodisperse population. The PDI was calculated using the following formula:

$$\mathbf{PDI = X_{50}- X_{10} / X_{90}}$$

where X_{50}, X_{10}, and X_{90} represent the particle sizes at 50%, 10%, and 90% cumulative distribution, respectively.

Normal gel preparation:

The 100 g gel formulation was prepared using the direct dispersion method. Propylparaben was dissolved in water at 80°C, while Carbopol 940 was accurately weighed and dissolved in water at 40°C, with continuous stirring for 30 minutes. A specified amount of **Millingtonia hortensis** extract was dissolved in a small volume of ethanol and then gradually added to the Carbopol solution, ensuring constant mixing throughout the process **[11]**.

Formulation of Ethosomal Gel

The ethosomal gel was prepared using the direct dispersion method with Carbopol 940 as the gelling agent. Carbopol 940 was dispersed in water at 40°C and stirred continuously for 30 minutes to ensure complete dissolution and uniform gel formation **[11]**.

Evaluation of Prepared Ethosomal Gel:

Measurement of pH:

The pH of the formulation was measured at room temperature using a calibrated electrode. The electrode was immersed in the preparation, and the pH value was recorded. A pH range of 4–6 was considered optimal for the formulation.

***In-vitro* drug release Study**

The permeation study was conducted using a modified Franz diffusion cell. Phosphate buffer (pH 7.4) was placed in the receptor compartment, while the formulations (suspension, ethosomal gel, and normal gel) were placed in the donor compartment. Goat skin was positioned between the receptor and donor compartments and securely clamped in place. The experiment was carried out for a duration of eight hours. At predetermined intervals, the receptor compartment was replenished with fresh phosphate buffer, and samples were collected. The drug content of the samples was immediately analyzed at 256 nm using a UV spectrophotometer **[12]**.

Drug content analysis:

A measured quantity of ethosomal gel was diluted to 10 mL using ethanol. The resulting mixture was sonicated for 20 minutes and then filtered. The drug content was quantified using methanol as a blank medium and analyzed at 256 nm using UV spectrophotometry **[13-19]**.

RESULTS AND DISCUSSION

Extract preparation:

Millingtonia hortensis leaves were air-dried and extracted using 50% ethanol at 75°C. For each batch, 20 grams of powdered plant material were used. The hydro-alcoholic extract was subsequently concentrated to one-third of its original volume using a rotary vacuum evaporator, yielding a final greenish-black extract.

Ethosomes preparation:

Nine formulations with varying concentrations of soy lecithin and ethanol were prepared using the cold method for ethosome formulation. Following preparation, the ethosomal formulations were optimized. Based on the entrapment efficiency results, the optimized batch B9 was selected for further evaluation. Zeta potential, a critical parameter influencing the stability of the formulation, was measured. All formulations, as shown in **Table 2**, exhibited negative zeta potential values, indicating the formation of stable ethosomal vesicles.

Organoleptic results: The obtained extract was a brownish-yellow in colour and aromatic in odour.

Zeta Potential and Particle Size: Zeta potential reflects system stability. More negative values (e.g., B9: -28.55 mV) indicate greater stability due to increased particle repulsion. Lower values (e.g., B3: -11.62 mV) suggest reduced stability and higher aggregation risk. Particle size affects drug delivery and absorption. Smaller particles (e.g., B3: 95.83 nm) enhance drug release but may reduce stability, while larger particles (e.g., B6: 411.55 nm) improve stability but slow drug release. Optimal sizes (100-300 nm, like B8: 228.57 nm) balance stability and delivery.

Determination of Entrapment Efficiency (EE): EE measures drug encapsulation within ethosomal vesicles. Higher EE (e.g., B9: 95.33%) indicates better drug retention, achieved with higher ethanol concentrations that enhance lipid bilayer fluidity. Lower EE (e.g., B1: 59.66%) reduces drug delivery efficiency.

Polydispersity index: The polydispersity index (PDI) was determined to assess the homogeneity of the formulation. Nine batches were evaluated for PDI, with higher values indicating a broad particle size distribution, while lower values suggest a more homogeneous ethosomal vesicle population **[15, 16]**. Lower PDI (e.g., B3: 0.09026) indicates a uniform size distribution, ensuring consistent drug release. Higher PDI (e.g., B7: 0.691) suggests uneven distribution, leading to inconsistent delivery.

The results for all parameters, including particle size, entrapment efficiency, zeta potential, and polydispersity index (PDI), for the nine batches are presented in **Table 2**.

As the concentration of alcohol increases, the zeta potential becomes more negative, which influences the entrapment efficiency of the ethosomal formulations. The percentage of drug entrapped was used to calculate the entrapment efficiency for each formulation. The higher drug retention observed in the ethosomal formulations may be attributed to the enhanced entrapment of **Millingtonia hortensis** leaf extract. The study indicated that the ethanol content in the ethosomal core plays a critical role in determining entrapment efficiency.

In the in-vitro release study, it was found that the permeation of intact vesicles into the stratum corneum may not be as significant as the penetration-enhancing effect of the ethosomal vesicles in promoting skin delivery under non-occlusive conditions.

The results demonstrated that batch B9, which exhibited the highest entrapment efficiency (95.33%), an optimal particle size (226.54 nm), a PDI value of 0.25, and a favorable zeta potential (-28.55 mV), is suitable for the preparation of ethosomal gel.

Table 2: Batch Specification of Different Formulation

Batches	Particle size	Entrapment efficiency	Zeta potential	PDI
B1	167.70	59.66	-18.26 mV	0.4730
B2	182.00	63.86	-15.48 mV	0.4600
B3	95.83	73.43	-11.62 mV	0.0902
B4	94.00	75.63	-16.10 mV	0.4730
B5	220.00	80.53	-24.13 mV	0.3500
B6	411.55	82.33	-18.39 mV	0.2693
B7	281.00	91.36	-16.81 mV	0.6910
B8	228.57	92.66	-22.60 mV	0.1943
B9	226.54	95.33	-28.55mV	0.2516

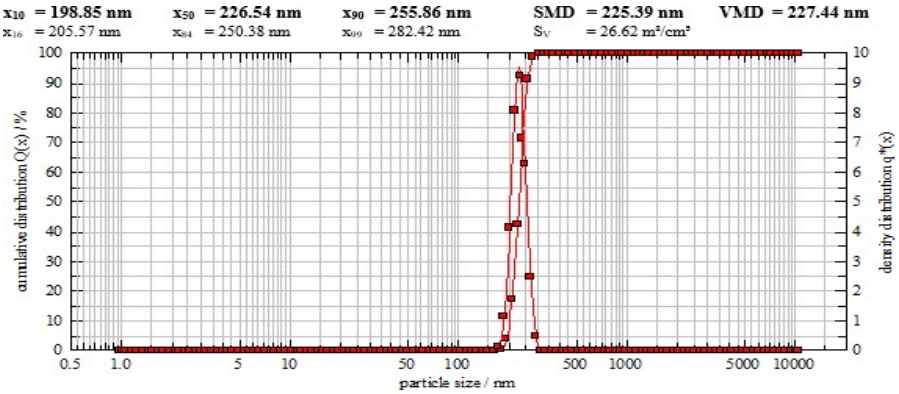

Figure 1: Particle Size of Optimised Batch (B9)

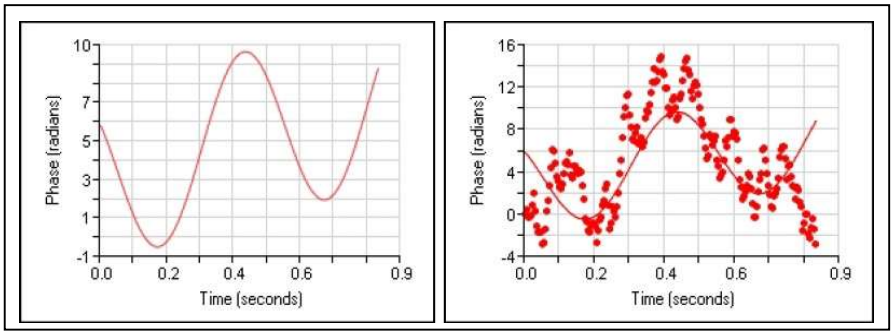

Figure 2: Zeta potential of batch b9

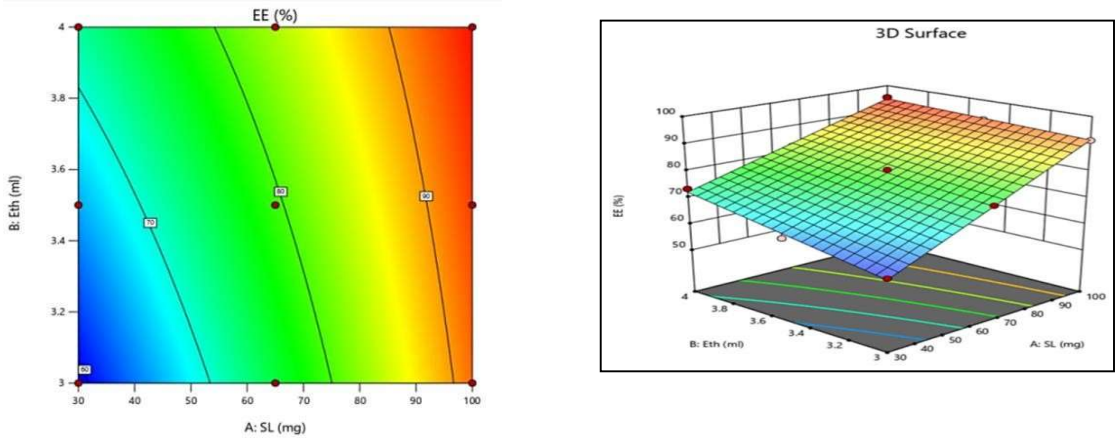

Figure 3: Effect of phospholipid and ethanol on entrapment efficiency

The effects of the amount of phospholipid (X1) and ethanol (X2) on the (Y1) on, entrapment efficiency were displayed by the response surface plot (3D) and the contour plot. As the concentration of ethanol and phospholipid increases, entrapment efficiency also increases.

Preparation of Ethosomal gel:

Carbopol polymer was used in 1% concentration with B9 Ethosomal solution to prepare the topical gel. The gel had a good appearance and was prepared correctly.

Additional gel preparation was done and its physicochemical characteristics, pH reading, drug content, and in vitro drug release investigations were examined.

Evaluation of Gel formulation: Formed ethosomal gel was evaluated for the different parameter.

Physicochemical Properties: The ethosome gel was found to be faint yellow. After visual inspection, faint yellow color was observed in the ethosome gel.

pH Measurement: pH of the gel was 5.7, which is suitable for human skin **[13]**.

Drug Content:

Drug content was found to be 91.76%

Comparative study of % drug release of normal gel, ethosomal suspension, & ethsomal gel:

In- vitro diffusion of normal gel, ethosomal solution, and ethosomal gel revealed a cumulative drug release of 8.80, 14.46, and 18.34%, respectively.

Table 3: Percentage drug release of normal gel, ethosomal suspension, & ethosomal gel

Time (hr)	% Drug release from ethosomal gel	% Drug release from normal gel	% Drug release from ethosomal suspension
1	0.6473	0.3276	0.5782
2	1.6921	0.7921	1.2289
3	3.4585	1.367	2.0157
4	5.7164	2.1506	3.4243
5	8.5769	3.3756	5.3776
6	11.2407	4.7032	7.4052
7	14.2802	6.3217	10.0743
8	18.3414	8.8046	13.7217

From this comparison, it was concluded that ethosomal gel shows a higher % of drug release than those of other transdermal formulations.

CONCLUSION:

It is always challenge to provide plant extract at high loading with effective skin penetration. A stable gel formulation was formulated and evaluated for topical delivery, utilizing ethosomes loaded with extracts from *M. hortensis* L. The study's findings suggested that the ethosomal carrier technology would work well with herbal extracts. In comparison to traditional gel, a stable topical gel containing ethosomes entrapped with *M. hortensis* leaf extract enhanced skin penetration and might be a potential solution for nascent researchers.

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Conflict of interests

The authors have no conflicts of interest regarding this investigation.

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