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ORIGINAL ARTICLE

A Comparative Approach to Evaluate diffusion Efficiency of Phytosomes and non-phytosomes form of Berberine chloride in *Punica granatum* Fruit peel Extract based topical gel

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ABSTRACT
Management of wounds and inflammatory skin conditions requires topical formulations that not only promote healing but also minimize irritation and inflammation. Natural bioactive compounds, such as berberine chloride and Punica granatum (pomegranate) fruit peel extract, have gained increasing attention due to their antimicrobial, antioxidant, and anti-inflammatory properties. However,berberine chloride's limited solubility and permeability restrict its therapeutic efficacy. This study compares the diffusion efficiency of phytosomal and non-phytosomal berberine chloride in topical gels with pomegranate peel extract, as combine therapeutic potential. Permeation studies were conducted with Franz diffusion cells employing synthetic dialysis membranes as well as goat ear skin membranes to mimic biological conditions. The release kinetics of berberine chloride and pomegranate extract were simultaneously analyzed using dual λmax spectrophotometry. Results demonstrated that the phytosomal gel significantly improved cumulative drug release, showing approximately 11% berberine chloride and 1% pomegranate extract release through the synthetic membrane and 27% and 16% release, respectively, through the goat ear membrane. In comparison, the non-phytosomal gel showed reduced permeation values with approximately 7% berberine chloride and 1% pomegranate extract release through the synthetic membrane, and 21% and 3% release, respectively through the goat ear membrane. Moreover, the phytosomal formulation exhibited enhanced flux, higher permeability coefficients, and shorter lag times, suggesting faster initial permeation followed by a more controlled, sustained release profile of metabolites. Overall, the findings confirm that phytosomes provide superior bioavailability and controlled delivery compared to conventional formulations, highlighting their potential as effective natural delivery systems for wound healing and anti-inflammatory therapy.
Keywords: *Phytosomes, Franz diffusion study, Berberine chloride, Pomegranate peel extract.*

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INTRODUCTION

Berberis aristata (Family: Berberidaceae) is a spiny, erect shrub that has been traditionally used in Ayurvedic and folk medicine systems for centuries. It is commonly known as Daruharidra, Darvi, and Hema Kanti in Sanskrit; Indian barberry and tree turmeric in English; Chitra and Kashmal in Hindi; and Churto and Chautari in Nepali. The plant is widely distributed in the Himalayan region and is considered a highly valuable medicinal shrub due to its potent pharmacological activities [1]. Among its parts, the root and stem bark are the richest sources of alkaloids, containing about 5% and 4.2% berberine, respectively [2]. Apart from berberine, they also contain several other important isoquinoline alkaloids such as palmatine, jatrorrhizine, berbamine, and columbamine, which contribute to the wide spectrum of medicinal activities attributed to the plant [3]. These bioactive molecules have been extensively studied and are reported to possess remarkable anticancer, antimicrobial, anti-inflammatory, antioxidant, and hepatoprotective properties [4]. Such therapeutic attributes provide strong scientific validation for the

traditional claims of Berberis aristata in treating liver disorders, promoting wound healing, and supporting its use as a well-known Ayurvedic medicine in India and other South Asian countries [5,6].

P. granatum (Family: Lythraceae), commonly known as pomegranate, is a shrub or small tree of immense nutritional and medicinal value [7]. In traditional systems of medicine, it is known as Dadima-Phalam in Sanskrit, Anar or Dhalim in Hindi, A Shih Liu in Chinese, Granaat in Afrikaans, Granatbaum in German, and Shajrataur-Rumman in Arabic [8]. The plant is native to Asian countries but has now been cultivated worldwide due to its high economic and medicinal importance [9]. Pomegranate is a rich source of polyphenolic compounds and secondary metabolites. The fruit peel, in particular, has been shown to contain 18–22% myrobalan-type tannins, along with flavonoids and anthocyanins [10]. Tannins are present in almost all parts of the plant, including dried fruit peel (up to 26%), root bark (28%), stem bark (10–25%), and leaves (11%), which make it a potent natural reservoir of bioactive constituents [11]. These phytochemicals have been characterized for their wide range of therapeutic properties, such as antidiarrheal, antioxidant, anti-inflammatory, antimicrobial, and wound-healing activities, further supporting their extensive use in traditional as well as modern herbal formulations [12, 13].

Wounds are defined as physical injuries that result in disruption of the normal structure and function of the skin. They may occur due to cuts, burns, accidents, surgical interventions, or underlying infections. The healing of wounds is a complex and dynamic biological process that involves several overlapping stages, including hemostasis, inflammatory response, tissue proliferation, and remodeling of the skin. While minor wounds usually heal naturally through the body's intrinsic repair mechanism, chronic or deep wounds require external interventions and palliative care. Millions of people across the globe suffer from chronic wounds, particularly those associated with diabetes, obesity, and vascular disorders, resulting in prolonged suffering and impaired quality of life. Furthermore, billions of dollars are spent annually on wound management and treatment worldwide, posing a significant economic burden on healthcare systems.

Currently, most of the widely available medicaments for wound healing contain synthetic corticosteroids, antibiotics, or antimicrobial agents. Although these formulations provide rapid symptomatic relief, their long-term use is associated with several complications, including microbial resistance, delayed wound contraction, skin thinning, allergic responses, and systemic side effects [14]. Therefore, there is a growing demand for alternative natural remedies and botanical-based formulations that can promote sustainable healing, improve skin repair, and minimize side effects during prolonged use. Medicinal plants enriched with bioactive phytoconstituents such as alkaloids, tannins, flavonoids, and polyphenols have gained much attention as safer and effective therapeutic agents for wound care [15].

In this novel research work, an attempt has been made to combine the therapeutic potential of two well-known natural agents, namely berberine chloride (derived from *B. aristata*) and pomegranate peel extract (*P. granatum*). Both agents possess proven wound-healing, antimicrobial, antioxidant, and anti-inflammatory activities. In order to enhance the bioavailability and skin permeability of berberine chloride, a phytosomal drug delivery system was developed. Phytosomes are lipid-compatible molecular complexes that improve the solubility, stability, and membrane penetration of plant-based bioactives, thereby enhancing their therapeutic efficacy. Here, berberine chloride was incorporated in phytosome form and further formulated into topical gels containing pomegranate extract. The comparative diffusion and release kinetics of phytosomal versus non-phytosomal forms were studied to evaluate the efficiency of drug delivery.

For this purpose, in vitro diffusion studies were carried out using synthetic dialysis membranes, while ex vivo studies were performed using goat ear skin membranes to closely mimic human skin conditions. The permeation of berberine chloride and pomegranate extract was simultaneously analyzed using dual λmax spectrophotometric methods. The release profiles obtained were further subjected to various kinetic models to understand the mechanism and pattern of drug diffusion. This comparative approach provides valuable insights into the potential application of phytosomal gel formulations for improved wound-healing and anti-inflammatory therapies.

MATERIAL AND METHODS

Chemicals and reagents

Berberine chloride (designated as S-BCl, Batch no. 633-65-8, Yucca Enterprises, Mumbai), Glycerin, Ascorbic palmitate, Sodium benzoate, Aloe vera gel, Disodium hydrogen phosphate, Potassium dihydrogen phosphate, Sodium chloride, N-hexane, Dichloromethane, Ethanol (95% pure), Distilled water.

Membranes for Franz diffusion study

Synthetic: Dialysis membrane (Dolphin Dialysis Membrane 70 (LA 393-1MT), pore size 2.4 nanometres, Mumbai), Biological: Goat ear skin membrane (collected from Slaughter house).

Instruments

Rotary evaporator (Roteva), UV visible spectrophotometer (UV-3200 Lab India), Franz diffusion apparatus (Dolphin).

Plant materials and extract preparation:

Pomegranate fruit peel powder (purchased from Dagduteli Kashtaushadhi shop, local market, Nashik) was extracted using hydro alcohol (ethanol: water, 60:40) by reflux method. The solution was then filtered and concentrated to obtain the pomegranate extract, designated as POM-EE.

Preparation of phytosomes:

Berberine chloride and soya lecithin in a 1:1.5 (250:500) ratio is mixed and 20 mL of dichloromethane added, followed by stirring at 40°C in rotary evaporator to form a thin film. To the flask few mL of n-Hexane was added, then the mixture was centrifuged and sediments are collected and dried. Further it is analyzed by DSC, SEM, Particle size, zeta potential and drug entrapment and loading.

Preparation of samples

Preparation of Phytosomes gel (designated PHY-G):

Different formulation batches were prepared, and one optimized batch was selected for the diffusion study. Following ingredients were used as Phytosomes of Berberine chloride-0.5 %, Pomegranate extract (POMEE)- 2 %, Glycerin-7 %, Ascorbic palmitate-0.1 %, Sodium benzoate- 0.1 %, in Aloe vera gel- (Q.S.), w/w

Preparation of non-phytosomes gel (designated as NPG):

It was prepared as Berberine chloride (designated as S-BCl)-5%, Pomegranate extract (POM-EE)- 2% mixed well in Aloe vera gel (Q.S.).

Preparation of Phosphate buffer pH 6.4

Disodium hydrogen phosphate-1.76 g, Potassium dihydrogen phosphate-1.36 g, and Sodium chloride-7.02 g. Mixed all chemicals in distilled water, and the volume was made up to 1000 ml. Adjusted the pH by using diluted NaOH or HCl [16].

Preparation of solutions for linearity

Determination of λ max and calibration curve of Standard Berberine chloride (S-BCl)

Standard Berberine chloride 5 mg was dissolved in 50 mL phosphate buffer pH 6.4 using a volumetric flask (100 ppm). From stock solution, 10, 20, 30, 40, and 20,30,40,50 ppm (10 mL) was prepared in stoppered test tubes. A spectral scan for 50 ppm was carried out between 200-700 nm in UV spectrophotometer and λ max was noted as 345 nm. Further absorbance was noted at 345 nm for 10 to 50 ppm. [17].

Determination of λ max and calibration curve of Pomegranate extract (POM-EE)

P. granatum fruit peel hydroalcoholic extract (POM-EE) 5 mg was dissolved in 50 mL phosphate buffer pH 6.4 using a volumetric flask (100 ppm). From stock solution, 10, 20, 30, 40, and 50 ppm volumes were prepared in mark stoppered test tube. From stock solution 10, 50 ppm (10 mL) were prepared in stoppered test tubes. A spectral scan for 50 ppm was carried out in between 200-700 nm in UV spectrophotometer, and λ max was noted as 403 nm. Further absorbance was noted at 403 nm for 10 to 50 ppm, [18].

The linearity curve developed for Berberine chloride and Pomegranate extract as Y axis - absorbance and X axis - conc. in µg, as shown in **(Figure 1 (a) and (b)).**

Membrane Preparation for Diffusion Study

Synthetic dialysis membrane: A dialysis membrane of 2 cm diameter was cut in a round shape and pre-soaked in phosphate buffer (pH 6.4) for 20 to 24 hours prior to use to ensure hydration and uniform penetrability.

Biological goat ear skin membrane: The goat ear skin was transported in saline solution, then thoroughly cleaned, shaved to remove hair and subcutaneous fat, and finally soaked in phosphate buffer (pH 6.4) for 30 minutes before mounting in the diffusion cells [19].

Franz Diffusion Cell Setup

For comparative study, the phytosomes gel (PHY-G) and non-phytosomes gel (NPG) were evaluated by Franz diffusion studies using both in vitro through synthetic dialysis membrane and ex vivo through goat ear skin membrane.

A total of four Franz diffusion cells were mounted for the study. Cells 1 and 2 were equipped with the synthetic dialysis membrane; cells 3 and 4 used goat ear skin as the biological membrane.

Each receptor compartment of the diffusion cells were filled with 30 ml of phosphate buffer (pH 6.4) and maintained at a constant temperature of 37 ± 0.5°C.
In the donor compartments, 1 g of gel was applied as follows:
a) Cell 1 contains non-phytosomes gel (NPG)
b) Cell 2 contains phytosomes gel (PHY-G)
c) Cell 3 contains non-phytosomes gel (NPG)
d) Cell 4 contains phytosomes gel (PHY-G)

Sampling and Simultaneous Analysis

The sample of 1ml was withdrawn at time intervals of 15, 30, 60, 120, 180, 240, 300 minutes from the receptor compartments of all four cells. Each withdrawn sample was immediately replaced with 1 mL of fresh phosphate buffer (pH 6.4) maintained at 37 ± 0.5°C to maintain sink conditions. The collected samples were transferred to stoppered test tubes and diluted up to 3 mL with phosphate buffer (pH 6.4).
The diluted samples were then analyzed spectrophotometrically at two wavelengths: 345 nm for Berberine chloride (S-BCl) and 403 nm for pomegranate extract (POM-EE), by setting dual wavelength mode to simultaneously quantify the number of metabolites diffused through the membranes over time [20].
Linearity equation as shown in (Figure 1 (a) and (b)), of standard Berberine chloride and Pomegranate extract in phosphate buffer are used to convert absorbance to concentration of withdrawn samples over the time, and further calculated cumulative drug permeated /cm^2 area of membrane. Comparative percentage cumulative drug release of two metabolites through both membranes are as shown in (Table 1) and graph plotted as, on x axis- time in minutes, on y axis- % cumulative drug release, as shown in (Figure 2 (a) and (b)).
Further comparative flux and permeability coefficient were determined.
Flux (J) is the rate at which a drug passes through a membrane per unit area, Flux (J) = Slope / Area in $mg/cm^2/hr$ and are calculated as using the slope of graph plotted as on x axis- time in hours and y axis- cumulative drug release in mg shown in (Figure 3 (a) and (b)) for both metabolites.
Permeability coefficient (Kp) represents the membrane permeability to the drug and is calculated as Kp = J / C_0 cm/hr., where C_0 is the initial drug concentration.
Secondly, Lag time which is the initial period before the drug starts to penetrate through the membrane at a consistent rate during diffusion, calculated with graph a.
Flux, permeability coefficient and lag time comparatively shown in (Table 2).
Further, diffusion kinetics were studied through various graphs plots for Zero order (x axis-% cumulative drug released, y axis- time), first order (x axis-log cumulative drug remaining, y axis-time), Higuchi (x axis-% cumulative drug released, y axis-square root of time), Hixson-Crowell (x axis-cube root of % drug remaining, y axis- time), and Korsmeyer-Peppas (x axis-log % cumulative drug released, y axis- log time). The best fit model was selected which is the most nearby value of R^2 to 1, are shown in (Table 3) and graphs were as shown in (Figure 4) and (Figure 5).

RESULTS AND DISCUSSION

This research paper major objective is for diffusion study so further elaborate results are discussed below, and phytosomes characterization are narrated in short as follows.
Berberine chloride phytosomes were analysed for DSC which shows endothermic transitions key peaks at 103.44°C, 193.26°C, and 241.58°C. Secondly the Particle size as 125.7nm and Zeta potential as +24.3mV, were found. Drug entrapment and loading calculated as 65%, and 103.93% respectively. Plain Berberine chloride was also analysed for Scanning Electron Microscopy (SEM) shows elongated, segregated, needle shape and crystalline form and secondly, SEM of phytosomes of Berberine chloride image at 2000x magnification shows spherical, moderately aggregated phytosomes with uniform distribution, hence it was proved that Phytosomes are successfully form.

Cumulative Drug Release (%CDR) determination

In (Table 1) and (Figure 2 (a) and (b)) comparative cumulative drug release (%CDR) of Berberine chloride phytosomal form PHY-G, exhibited the highest % CDR (~27%) through biological and (~11%) through the synthetic membrane. While non phytosomal formulation of it in NPG form showed quite moderate value (~21%) through biological and (~7%) through synthetic barrier. While metabolites of pomegranate extract (POM-EE) in PHY-G formulation, has shown moderate %CDR (~16%) through biological and very low (~1%) through synthetic membrane, while in NPG formulation, of it exhibited minimum release (~1 and ~3%) on both synthetic and biological membrane at 300 minutes.
The % CDR, flux, and permeability coefficient results clearly demonstrate that the PHY-G formulation significantly exhibited the highest transdermal delivery of berberine chloride phytosomes and

pomegranate extract across biological membranes, which more closely resemble in vivo skin conditions compared to synthetic membranes. This indicates that the PHY-G formulation can effectively enhance the penetration of both metabolites through the skin, making it a promising approach for topical therapy. When comparing the phytosomal form with the non-phytosomal form, it is evident that the phytosomes exhibited a markedly higher release profile. This enhanced permeation can be attributed to the ability of phytosomes to improve the solubility, stability, and membrane affinity of the bioactive compounds, facilitating better drug transport across the lipid-rich stratum corneum.

Furthermore, the inclusion of pomegranate extract alongside berberine chloride phytosomes in the PHY-G formulation resulted in a synergistic effect, increasing the overall release and permeation of both metabolites. The reduced lag time observed for both bioactive compounds in the PHY-G formulation suggests rapid initial availability, which is essential for achieving a prompt therapeutic effect, followed by a sustained release over an extended period. This indicates that the novel phytosomal formulation successfully promotes improved transdermal diffusion, combining immediate action with prolonged drug availability.

In kinetic modeling, the diffusion data reveal that when berberine chloride passes through a synthetic barrier, the release follows the Hixson–Crowell model, indicating an erosion-controlled mechanism. This results in an initially faster release that gradually slows as the drug diffuses out of the matrix. In contrast, when berberine chloride and pomegranate extract are delivered via the PHY-G formulation through biological membranes, the first-order kinetic model provides the best fit, reflecting a concentration-dependent release with an initially rapid phase followed by slower diffusion over time. Meanwhile, the release of pomegranate extract in both PHY-G and NPG formulations adheres to the Korsmeyer-Peppas model, indicating diffusion-controlled kinetics involving a combination of drug diffusion and gel swelling, which ensures a slow, controlled, and sustained release.

Overall, these results revel the therapeutic potential of phytosome-based gels in enhancing the skin permeation of berberine chloride and pomegranate extract. The PHY-G formulation demonstrates superior efficacy on goat ear skin, which closely mimics human skin, highlighting its role as a natural and scientifically sound platform for topical drug delivery. This strategy offers significant potential for optimizing skin-targeted treatments, improving dermal bioavailability, and potentially enhancing clinical outcomes in the use of herbal therapeutics.

Table 1: Comparative %CDR between PHY-G and NPG at λ max 345 nm and 403 nm (Berberine chloride analysis, POM-EE analysis respectively)

345 nm (Berberine chloride analysis)				
	Dialysis membrane		Goat ear skin membrane	
Time in minutes	Cell 2	Cell 1	Cell 4	Cell 3
	PHY-G	NPG	PHY-G	NPG
15	2.168	2.662	9.402	9.342
30	5.474453	5.338204	20.0146	18.88905
60	8.693431	5.290511	22.45839	19.28321
120	10.32263	5.306569	24.16204	19.29993
180	10.6073	5.916832	25.60292	19.31
240	11.14161	7.410175	26.15912	20.49197
300	11.153	7.424672	27.84088	21.05255
403 nm (POM-EE analysis)				
15	0.0605	0.89	4.554	1.043
30	0.302804	1.673832	10.08785	2.083178
60	1.025701	1.014579	10.65421	2.198131
120	1.449533	1.090654	11.01869	2.219626
180	1.519626	1.131308	12.72897	2.336916
240	1.546262	1.228037	13.50841	2.652336
300	1.561869	1.230841	15.57757	2.774299

*CDR- cumulative drug release, PHY-G: Phytosomes gel, NPG- Non-Phytosomes gel, POM-EE- Pomegranate extract

Flux, Permeability Coefficient (Kp), and Lag Time determination:

As shown in **(Table 2)**, Berberine chloride phytosomes in formulation PHY-G, and for Pomegranate extract when it is combination with phytosomes in PHY-G, exhibited higher flux and permeability coefficient in both membrane and always found highest in biological membrane. And its lag time is shorter in biological membrane and even shortest with phytosomes form.

Table 2: Comparative Flux(J), Permeability coefficient (Kp) and lag time between Phytosomes gel (PHY-G) and Non-Phytosomes gel (NPG)

Membrane	Formulation	Flux (mg/cm^2/hr.)	Permeability Coefficient, Kp (cm/hr.)	Lag time (min.)
345 nm λ max (Berberine chloride analysis)				
Dialysis	Phytosomes gel (PHY-G)	0.02857	0.00575	30
	Non-phytosomes gel (NPG)	0.00809	0.00162	30-45
Goat ear skin	Phytosomes gel (PHY-G)	0.06904	0.01381	0-10
	Non-phytosomes gel (NPG)	0.00876	0.00175	10-20
403 nm λ max (POM-EE analysis)				
Dialysis	Phytosomes gel (PHY-G)	0.01538	0.00308	30-45
	Non-phytosomes gel (NPG)	0.00099	0.0002	30-60
Goat ear skin	Phytosomes gel (PHY-G)	0.01965	0.00393	10-20
	Non-phytosomes gel (NPG)	0.01073	0.00215	20-30

* PHY-G: Phytosomes gel, NPG- Non-Phytosomes gel, POM-EE- Pomegranate extract

Kinetic models:

As per (Table 3) and (Figure 4) and (Figure 5), the diffusion kinetics indicate that the Hixson–Crowell model best describes the release of Berberine chloride from PHY-G and NPG through the synthetic membrane. In contrast, a first-order release model best fits the release of Berberine chloride and pomegranate extract from PHY-G and NPG through the biological membrane. While Pomegranate extract from PHY-G and NPG followed the Korsmeyer–Peppas model on dialysis membrane.

Table 3: Comparative kinetic models of dialysis membrane and goat ear skin membrane between PHY-G and NPG at λ max 345 nm and 403 nm

Membrane	Formulation	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)	Kors-Peppas (R^2)	Hixson-Crowell (R^2)	Best Fitting Model
345 nm λ max Berberine chloride							
Dialysis	PHY-G (cell 2)	0.6112	0.6118	0.5136	0.4098	0.6171	Hixson-Crowell
	NPG (cell 1)	0.6794	0.6851	0.5614	0.4904	0.7479	
Goat ear skin	PHY-G (cell 4)	0.6006	0.6265	0.4885	0.3730	0.6177	First order
	NPG (cell 3)	0.5227	0.5338	0.3963	0.3462	0.5300	
403 nm λ max POM-EE							
Dialysis	PHY-G (cell 2)	0.5678	0.568	0.6176	0.8863	0.6766	Kors-Peppas
	NPG (cell 1)	0.4426	0.4424	0.4877	0.9706	0.5989	
Goat ear skin	PHY-G (cell 4)	0.7387	0.7568	0.6093	0.4399	0.7508	First order
	NPG (cell 3)	0.6449	0.6474	0.5145	0.5372	0.6465	

* PHY-G: Phytosomes gel, NPG- Non-Phytosomes gel, POM-EE- Pomegranate extract

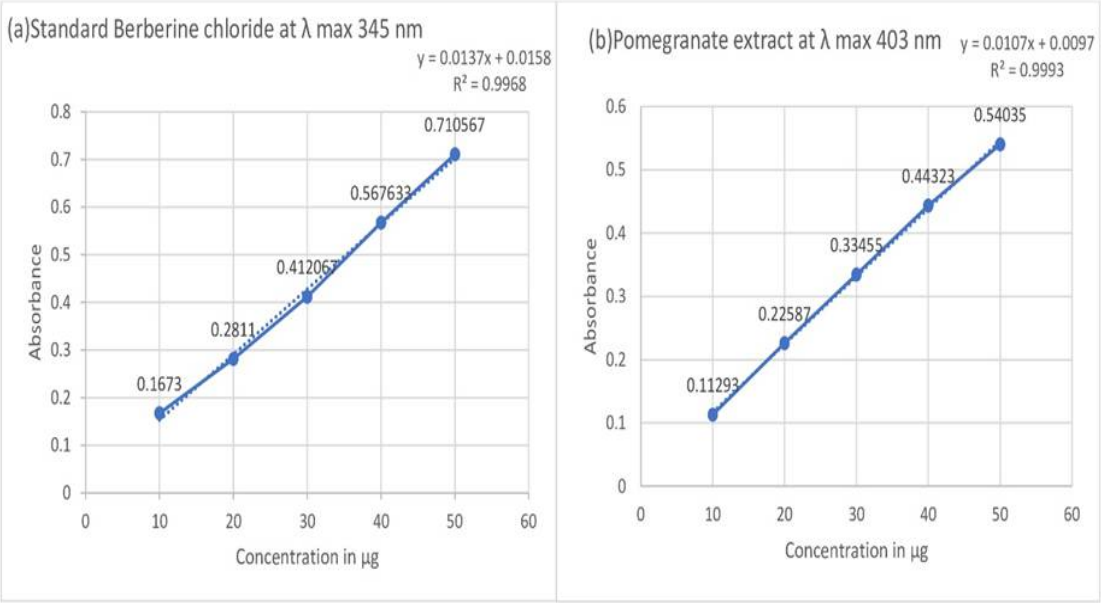

Figure 1: Linearity in Phosphate buffer pH 6.4 (a) Standard Berberine chloride at λ max 345nm, (b) Pomegranate extract at λ max 403 nm

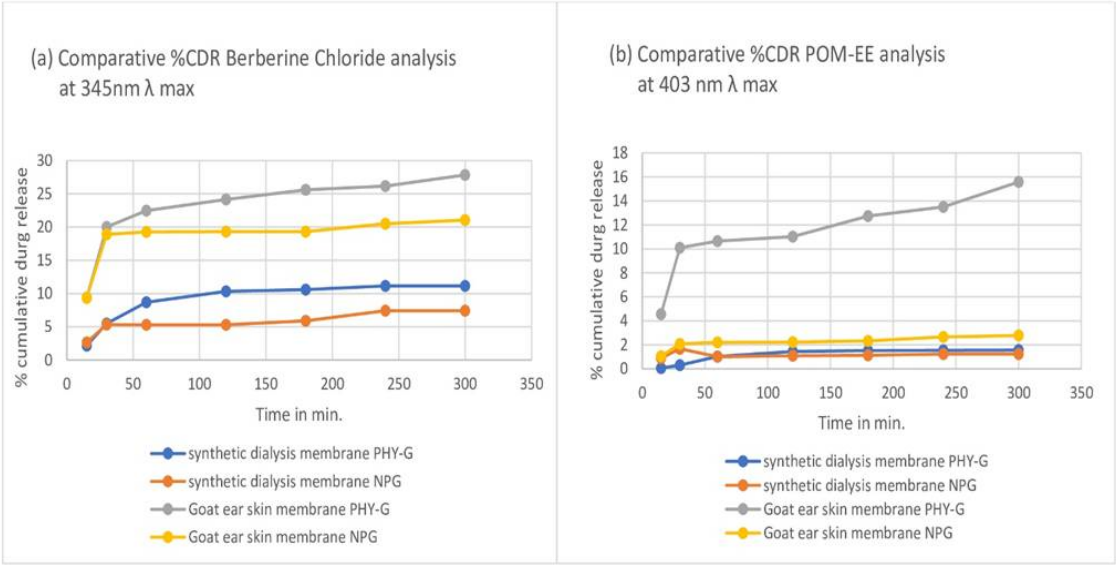

Figure 2: Comparative % CDR through synthetic dialysis membrane and goat ear skin membrane between PHY-G and NPG (a) Berberine chloride analysis at 345 nm λ max, (b) POM-EE analysis at λ max 403 nm

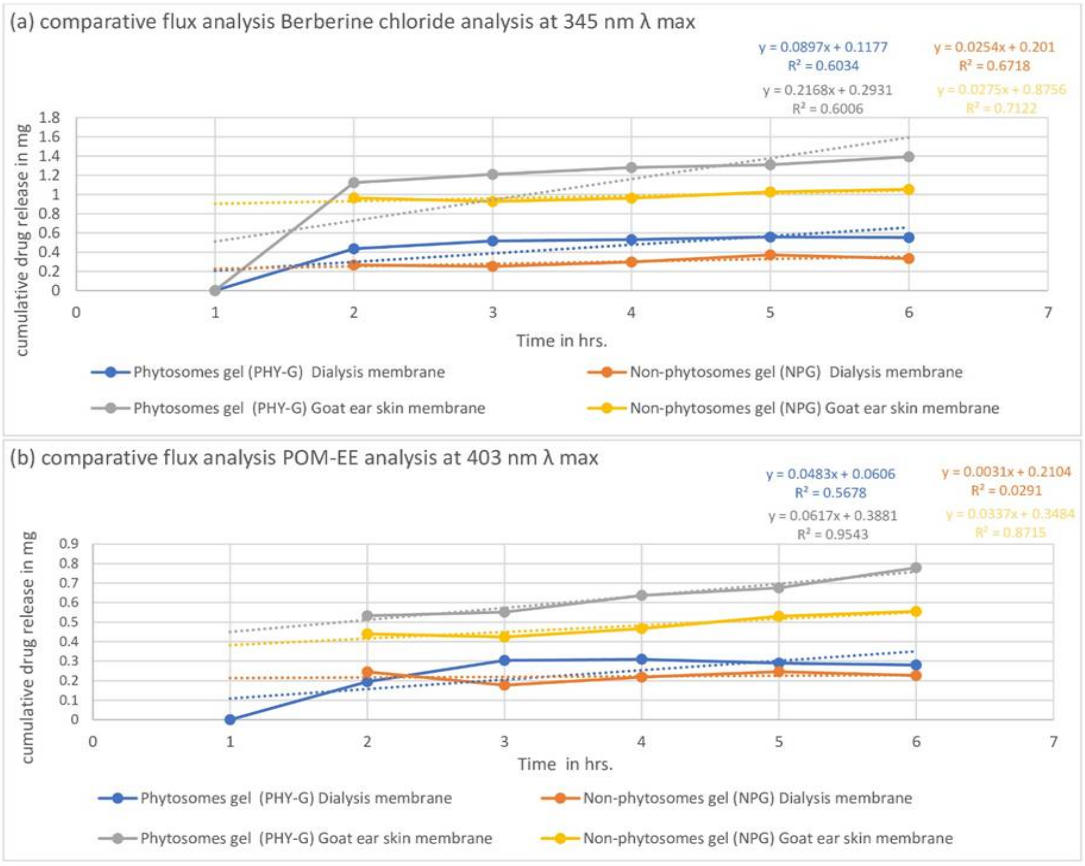

Figure 3: Comparative Flux analysis of Phytosomes gel (PHY-G) and Non-phytosomes gel (NPG) through dialysis membrane and goat ear skin membrane (a) Berberine chloride analysis at 345 nm λ max, (b) POM-EE analysis at 403 nm λ max

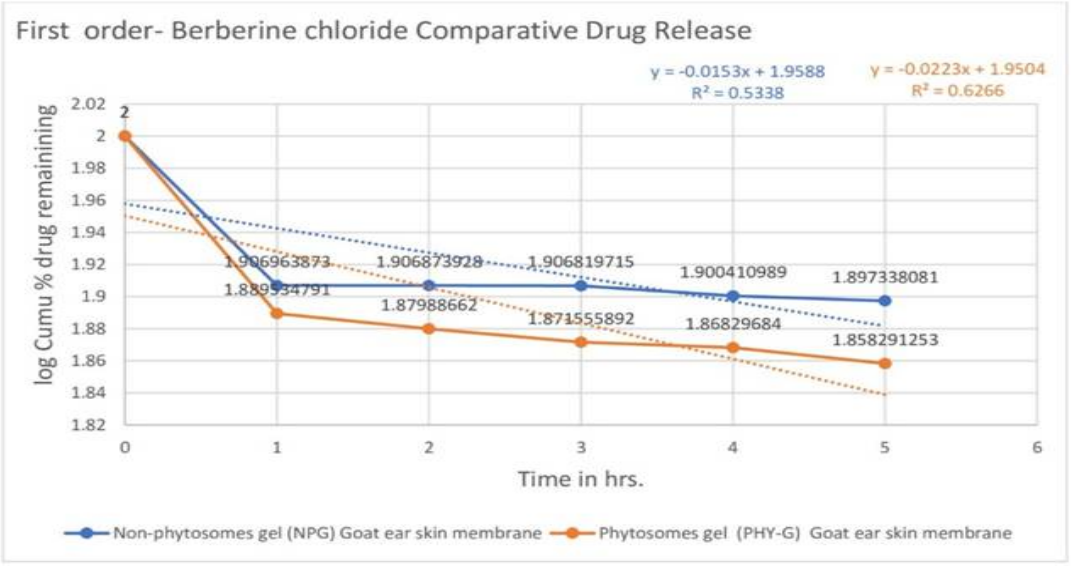

Figure 4: Comparative kinetic modelling for Franz diffusion study in goat ear skin membrane- between Phytosomes gel (PHY-G) and Non-phytosomes gel (NPG) for permeability of Berberine chloride at λ max 345 nm

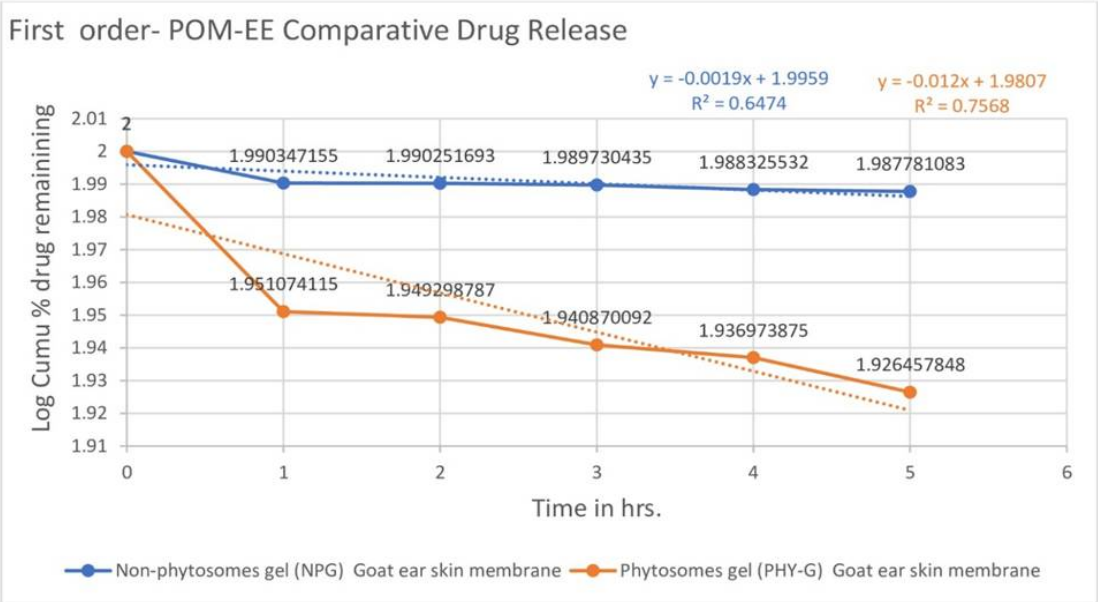

Figure 5: Comparative kinetic modelling for Franz diffusion study in goat ear skin membrane- between Phytosomes gel (PHY-G) and Non-phytosomes gel (NPG) for permeability of POM-EE at λ max 403 nm

CONCLUSION

The study finds that the phytosomes gel containing Berberine chloride with P. granatum extract improves skin permeation and drug release in comparison with non-phytosomal gel. it also exhibited in increased flux, enhanced permeability, and shorter lag time, particularly across the goat ear skin membrane. This highlights to its effectiveness in enhancing transdermal diffusion and along with sustained released of bioactive compounds for topical use.

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