RESEARCH PAPER

Design and Optimization of Ipomoea cairica (Railroad Creeper) Extract-Loaded Phytosomes for In-Vitro Anti-Inflammatory Potential Using Box-Behnken Design

G. Gnanarajan[1], Jyoti Joshi[2*], Shaweta Sharma[3]

[1]Department of Pharmaceutics, School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand - 248001, India

[2*]Research Scholar, Department of Pharmaceutics, School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand - 248001, India. Email: jpandey1112@gmail.com; Contact: 9634705738 (Corresponding Author)

[3]Department of Pharmacy, School of Medical & Allied Sciences, Galgotias University, Greater Noida, UP, India

ABSTRACT

Introduction: This study aims to develop and optimize a phytosome-based drug delivery system encapsulating the hydroethanolic extract of Ipomoea cairica (IPc-ex) to substantiate its traditional applications in treating inflammation.

Material and Methods: The crude plant material was subjected to extraction using various solvents including hydroethanolic, chloroform, ethyl acetate and n-hexane. Among these, hydroethanolic solvent yielded the highest extraction efficiency. IPc-ex-PS were formulated using the thin film hydration method. The Box-Behnken design was utilized to optimize the formulation and process parameters. Independent variables were taken as Phospholipid concentration (A), temperature (B) and reaction time (C) while dependent variables were Particle size and % Entrapment efficiency. Characterization of the phytosomes was conducted using zetasizer, FT-IR and TEM analyses. The in-vitro drug release from IPc-ex phytosomes was assessed using dialysis bag technique. The anti-inflammatory effect of hydroethanolic extract (IPc-ex) and hydroethanolic extract loaded phytosomes (IPc-ex-PSopt) was evaluated through the protein denaturation study.

Results: The phenolic content was found to be 43.15±1.28 mg Gallic acid equivalent/g and flavonoid content was 26.39±1.56 mg Quercetin equivalent/g in hydroethanolic extract of Ipomoea cairica. The optimized batch of hydroethanolic extract loaded phytosomes (IPc-ex-PSopt) exhibited the smallest average size of 118 nm. The entrapment efficiency and zeta potential of 79.59±0.21% and -25.8mV, respectively. The percentage drug release of the active constituent from IPc-ex-PSopt was 87.75±1.27% at the end of 12 hr. The optimized formulation (IPc-ex-PSopt) exhibited a concentration-dependent inhibitory effect with a maximum inhibition of 87.49±0.79% at 200 µg/ml and 37.61±0.75% at 50 µg/ml significantly when compared with standard drug, Diclofenac sodium.

Discussion: The findings demonstrate that Ipomoea cairica extract-loaded phytosomes (IPc-ex-PSopt) can serve as an effective delivery system with high encapsulation efficiency and controlled release.

Conclusion: The study suggests that IPc-ex-PSopt exhibit potential efficacy in mitigating inflammatory conditions, as evidenced by these experimental results.

Keywords: Anti-inflammatory, Ipomoea cairica, phytosomes, Box-Behnken design

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Introduction

The applications of herbal plants and their secondary compounds in disease management has gained increasing prominence as a form of complementary medicine [1]. Inflammation is the body's defence mechanism in response to potentially harmful stimuli such as allergens or tissue injury [2]. However, uncontrolled inflammatory responses are the primary cause of a wide range of disorders, including cardiovascular abnormalities, allergies, metabolic syndrome, autoimmune diseases and cancer, imposing a significant financial burden on both individuals and society as a whole [3-5]. Various pharmaceutical interventions, including corticosteroids, non-steroidal anti-inflammatory agents and immunosuppressive medications are employed to modulate and attenuate inflammatory responses [6]. Nevertheless, it is crucial to acknowledge that these therapeutic interventions are not devoid of potential adverse effects [7]. Rheumatoid arthritis andother chronic inflammatory diseases remain among the most prevalent health concerns globally [8]. Despite the dominance of synthetic pharmaceuticals in the current market, the potential for adverse effects persists. Prolonged utilization of these agents may result in significant adverse effects upon chronic dministration, with gastrointestinal disturbances being the most prevalent.

In contrast, our objective in clinical practice is to utilize the minimum number of pharmaceutical agents necessary while simultaneously achieving optimal levels of efficacy and safety. Consequently, it is necessary to utilize natural anti-inflammatory agents to enhance pharmacological efficacy and mitigate adverse effects associated with prescription medications. Despite its status as an undocumented scientific discipline, herbal medicine is firmly established in certain cultures and traditions, currently serving asa primary healthcareapproachfor approximately 80 percent of individuals residing in rural areas [9]. Recent studies have reported anti-inflammatory effects in numerous herbs, including *Ipomoea* species, *Passiflora* species, *Curcuma longa*, *Rosmarinus officinalis*, *Oenothera biennis*, *Borago officinalis*,*Zingiber officinale*, and *Harpagophytum procumbens*[10].

Ipomoeacairica, belong to Convolvulaceae family, is commonly referred as “MorningGlory” or “Rail-RoadCreeper.” This plant species originates from the eastern Mediterranean region and tropical Africa. Traditionally, it is used in Brazillian, Indian, and Southeast Asian medicine for treating rheumatism, It has been widely disseminated and cultivated across tropical Asia, including Philippines and various Pacific Islands, as well as South America[11]. *Ipomoea cairica* contains bioactive phytochemicals such as flavonoids, alkaloids, steroids and triterpenoids demonstrated to exert antioxidant and anti-inflammatory effects by reducing mediators like histamine, pro-inflammatory cytokines and oxidative stress markers [12-13].

These anti-inflammatory properties have a biochemical basis, as *Ipomoea cairica* extracts inhibit key enzymes and mediators involved in inflammation i.e., COX-2, iNOS and TNF-α [14]. *Ipomoea cairica* leaves are potential as a source of antimicrobial and antioxidant phytochemicals that could support natural preservation in foods and multiple biomedical uses, though they are not established food ingredients and require safety evaluation before food applications. Recent studies report antibacterial constituents and broad bioactivities that justify interest in functional formulations and topical or adjunct biomedical uses rather than direct dietary use at this time [15-16].

Leaf and aerial-part extracts inhibit common food-relevant bacteria, with zones of inhibition against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella typhi*, and the isolated triterpenoid friedelin showing MICs of 125–250 μg/mL, supporting potential roles as

natural antimicrobials or active-packaging additives pending safety and sensory assessment [16]. Qualitative phytochemical profiling confirms phenols, flavonoids, tannins, saponins, and other antioxidant classes in leaves, aligning with potential antioxidant preservation functions to slow lipid oxidation or color loss in food systems if validated in food matrices

[17]. A 2024 study isolated friedelin from I. cairica aerial parts and demonstrated antibacterial activity (e.g., MIC 125 µg/mL against E. coli), supporting development of topical antimicrobials or adjuvants targeting Gram-negative and Gram-positive pathogens[16]. Ethanolic extracts show antinociceptive activity with effects in the inflammatory phase of the formalin test in mice, indicating anti-inflammatory potential consistent with traditional use for rheumatism and inflammations[14].Nonvolatile ethanolic extracts and a dichloromethane fraction exhibit potent larvicidal activity against Aedes aegypti (e.g., LC50 ≈ 42.1 mg/L for crude stem extract and 30.6 mg/L for the DCM fraction), supporting vector control applications relevant to public health[18].

Leaves contain multiple bioactive classes (alkaloids, flavonoids, phenolics, tannins, saponins, terpenoids, anthraquinones, and glycosides), providing mechanistic bases for antimicrobial and antioxidant effects observed *in-vitro*[17].Variouscomponents like luteolin, isoluteolin, rutin, quercetin, methylbenzoate, methylsalicylate, lemolin etc.have been isolated from aerial parts and root region of *Ipomoea cairica* plant, respectively. Different extracts have been documented to exhibit a broad spectrum of biological activities, such as antibacterial, antispasmodic, antifungal, antiviral, and protein synthesis inhibitory properties[19].The anti-inflammatory properties of alcoholic and petroleum ether extracts of leaves have already been demonstrated[14, 21]. Different studies have reported that hydroalcoholic (aqueous methanol) extract of Ipomoea cairica leaves shows strong anti-inflammatory effects in various experimental models, such as formalin-induced inflammatory phase test in mice, exhibiting dose-dependent reduction of inflammation [14, 19].

Phytosomes can significantly enhance the transdermal delivery of plant extracts by improving the absorption, bioavailability, and skin penetration of phytoconstituents. This is achieved through their unique structure, which is capable of overcoming the skin's natural barrier and ensuring effective release of active plant compounds into deeper skin layers [21-22]. Phytosomes are vesicular systems formed by complexing plant extracts with phospholipids (usually phosphatidylcholine). This complexation increases the lipophilicity of hydrophilic phytoconstituents, allowing them to more readily integrate with the lipid-rich environment of the stratum corneum, the primary barrier for transdermal drug delivery [22-23]. The key mechanisms include enhanced entrapment and stability of plant actives due to strong interaction with phospholipids, better penetration through the stratum corneum via intercellular and intracellular pathways and improved skin hydration, enzyme balance, and collagen structure, further facilitating penetration [23-24]. Phytosomes increase percutaneous (transdermal) absorption by acilitating transport across the skin's outer layer. They show greater bioavailability and therapeutic efficacy by protecting actives from degradation and improving their pharmacokinetics and pharmacodynamics.

Box-Behnken design (BBD) is a response surface methodology design that requires fewer experimental runs compared to full factorial designs while effectively exploring interactions between variables [25-26]. It allows study of three-level factors (low, medium, high) and captures linear, quadratic, and interaction effects, making it suitable for optimizing multiple formulation/process variables [27]. The design avoids experiments at extreme corners where all factors are at their highest or lowest levels simultaneously, which can be infeasible or unsafe in formulations [25]. It supports simultaneous optimization of several critical variables impacting quality attributes like particle

size, entrapment efficiency, and bioavailability in drug delivery systems such as phytosomes [28].

BBD generates data amenable to analysis of variance (ANOVA) and regression modeling, aiding in identifying significant factors and predicting optimal conditions. BBD efficiently estimates first-order (linear), second-order (quadratic), and interaction effects among factors, providing a detailed understanding of variable effects on responses [27]. Different species of *Ipomoea* have been investigated by various researchers, however, the hydroethanolic extract of *Ipomoea cairica* has not been evaluated for anti-inflammatory activity after loading in phytosomes. Thus, the present study aimed at development and optimization of phytosome formulations loaded with *Ipomoea cairica* leaves extract using the Box-Behnken design approach, to enhance the extract's permeability, stability, and anti-inflammatory efficacy in-vitro by systematically investigating formulation variables to achieve optimal particle size, entrapment efficiency, and other physicochemical properties for effective anti-inflammatory activity.

Material and Method

Materials

Standard Quercetin and Dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich, USA. Cholesterol, dichloromethane, ethanol, and Glyceryl monostearate were purchased from Merck, Japan.

Plantmaterialcollection

Mature fresh plant leaves and stem of *Ipomoea cairica* were gathered for the raw plant material from Rajeswari Nursery in Doiwala, Dehradun Aug, 2022. After that the leaves were fully dried, the entire plants pecimen's herbarium was sent to Botanical Survey of India (BSI), a division of the Forest Research Institute in Dehradun that specializes in botany, for taxonomic identification of plant species.*Ipomoea cairica* as identified, and voucher specimen number for it was BSI/NRC/Tech./Herb (Ident.)/2022-23/1190. The fresh plant leaves were rinsed with water, dried in shade, coarsely powdered, and stored in an airtight container until used. This crude material further utilizes for the extraction.

Macroscopical and Microscopic examination

Macroscopic parameters of *Ipomoea cairica* leaves were determined by analyzing organoleptic properties such as color, odor, shape, taste and surface texture. Microscopic analysis of the fine powder of *Ipomoea cairica* leaves was carried out by evenly spreading a thin layer of the powdered drug on a clean, dry glass slide, treating it with prescribed reagents, and observing it under a microscope to examine various characteristics of the sample[29]. The microscopical characteristics of *Ipomoea cairica* plant were shown in results and discussion section.

Preparation of *Ipomoea cairica* Plant Extract

For the preparation of *Ipomoea cairica* extract,the crudecoarse powder of leaves was used.For the same, about 25 g dried whole plant powder was then subjected to extraction with different solvents like Petroleum ether, Hydroethanolic, Acetone, Ethyl acetate, Chloroform and n-hexane, respectively and extraction was conducted with the help of Soxhlet apparatus.The powder sample-to-solvent ratio was kept as 1:20which, often gives good extraction yield and efficiency, especially for phenolic and flavonoid compounds[30].

Additionally, extracts were filtered and then concentrated using a rotary evaporator at 40-50 ^{0}C with approx. 100-200 mbar vacuum using a chiller set cold enough to fully condense solvent and 100-150 rpm rotation which is to be fine tuned to balance evaporation and condensation without bumping or overheating sensitive phytochemicals [31].The extract was dried using vacuum desicator to get a consistent weight with a satisfactory extractive value [32].

Determination of Extractive value

The determination of extractive value is essential for assessing the nature of chemical constituents and evaluating the concentration of specific

components in the solvent used during the extraction process.In this technique, the various extracts of *Ipomoea cairica* were filtered and evaporated until dry, with traces of crude plant material accumulating at China dish bottom at 50°C. Extractive value of different solvent extracts was determined [33].

Ash Value

Ash value is an inorganic residue remaining after a drug or herbal product has been incinerated at a high temperature. It is a measurement of the product's total mineral content, and utilized to assess its purity, safety and quality.

a) Total ash value: In a crucible, 1g of drug was taken and ignited at a temperature of 600-700ºC. After 5 minutes, the ash was removed and weight was determined until it remained constant. Drug's air-dried total ash value was estimated.

b) Water soluble ash: Initially convert drug into ash and boil it for 5 minutes with distilled water (25 ml). It was washed thoroughly and weighed. The ash weight isdeducted from insoluble elements weight. Proportion of water soluble ashes in mixture was determined.

c) Acid insoluble ash: The medication should first be reduced to ash and boiled for 5 minutes in diluted hydrochloric acid (25 ml). After gathering material in the crucible, give it a thorough water wash. Weigh the content and determine how much acid insoluble ash present in the mixture[33].

Foreign Organic matter analysis

Foreign organic matter (FOM) analysis is a process for identifying and quantifying organic contaminants in a sample. To get rid of the dust particles present on leaf or fruit surface, entire plant was washed completely and individually with water. The gathered material was spread out on filter paper, allowing any extra water to run out. Then 500 g of the thoroughly cleaned and drained whole plant was collected and applied to a white, spotless muslin cloth in a thin layer. By utilizing a 6x magnifying lens and visual inspection, foreign objects were separated. Weighing the sections of the sorted foreign matter, we determined the amount of foreign matter present in grams per 100 grams of the sample [34]. There were three sets in all where the method was used. Formula to calculate the foreign organic matter are shown in equation.

$$\% \text{ Foreign Organic Matter} = \frac{(M_1 - M) \times 100}{M_2}$$

M= Empty dish weight in gram

M_1= Dish with foreign matter weight in gram

M_2= Sample weight (whole plant material) in gram

Fluorescent analysis

Fluorescent analysis is a type of analytical chemistry that uses the phenomenon of fluorescence to identify and quantify substances. A molecule can undergo fluorescence when it absorbs light with a given wavelength and then releases light with a longer wavelength. Fluorescence can be used to distinguish between various molecules since the wavelength of light emitted by each molecule is unique.In the fluorescence study, powdered drug material was treated with different chemical reagents HCl, dilute HNO_3, dilute H_2SO_4, sodium hydroxide, antimony trichloride and was observed under visible, UV and ordinary light, which shown predominantly fluorescence effect in extract of *Ipomoea cairica* [34].

Loss on drying

A quantitative analytical method called loss on drying (LOD) is used to calculate how much volatile matter and also the moisture content present in a sample. Precisely weighed 1 g sample was dried in hot air oven at 100°C till a consistent weight of powder was attained and the loss on drying was calculated [34]. Loss on drying and moisture content were calculated by the following formulae:

$$\% \, Loss \; of \; drying = \frac{Weight \; lost \; after \; drying}{Total \; weight \; before \; drying} \times 100$$

Phytochemical analysis

The process of determining and measuring the secondary metabolites contained in diverse plant extracts is known as phytochemical screening. It is a first stage in the process of finding new drugs and is meant to evaluate a plant's possible medicinal value.

By employing traditional methods for confirmatory qualitative phytochemical screening of plant extracts, the main chemical classes (tannins, phenols, alkaloids, glycosides, terpenoids, and steroids) present in the extracts were identified.The different extracts of plant *Ipomoea cairica* (IPc) with several solvents were characterized and findings were shown in results and discussion section.The extract was dried in a vacuum desiccator to achieve a consistent weight, and the extractive value was measured.The extract which has shown good extractive value was selected further for the formulation of dosage form [35].

Characterization of *Ipomoea cairica* extract (IPc-ex)

The *Ipomoea cairica* extract (IPc-ex) was prepared using the afore mentioned method and subsequently freeze-dried at −100 °C for approximately 5 days using a ScanvacCoolSafe Touch 100-9 system (Labogene). The resulting freeze-dried IPc-ex was stored at −20 °C until further use. It was then analyzed for Quercetin, total phenolics (TP), and total flavonoids (TF).

Quercetin Content determination:

The Quercetin content was quantified using method given by Srivastava N. et al., (2024). Initially, sample solutions of drug (Quercetin) were made in the concentration having a range of linearity from 2-10 µg/mL at a selected wavelength of 376 nm [36].

Thin Layer Chromatography (TLC) Fingerprinting analysis of hydroethanolic extract of *Ipomoea cairica* (IPc-ex) leaves:

TLC is a simple and effective technique commonly used to identify and quantify secondary metabolites in herbal extracts. In this study, TLC fingerprinting of the herbal extract was carried out using Silica gel 60 F254 (Merck) plates as the stationary phase to determine the number of components present, identify specific compounds, and assess the chemical purity. Additionally, TLC can be employed to monitor reaction progress by observing the formation of products or the disappearance of reactants[13, 18]. Table 1 showed varied ratios of the solvent system listed below, were used to perform the TLC analysis.

Table 1: Solvent system in different ratio for analysis of TLC of hydroethanolic extract of *Ipomoea cairica* (IPc-ex)leaves

S. No	Solvent system	Ratio
1	Toluene: Ethyl acetate	5:5
2	Toluene: Ethyl acetate	6:4
3	Toluene: Ethyl acetate	7:3
4	Toluene: Ethyl acetate	8:2

TP content determination

For the total phenolic content, a 20 mg sample of the extract was prepared in 50 ml of distilled water, and 1 ml of this preparation was mixed with 1 ml of Folin-Ciocalteu reagent. The mixture was vortexed and, after a 3-minute interval, 1 ml of a 20% sodium carbonate solution was incorporated and incubated for 1 hour. Absorbance of the resultant color was measured at 760 nm using a UV-Visible Spectrophotometer (Shimadzu UV1800 Spectrophotometer). The total phenolic content was calculated from a Gallic Acid standard curve and reported as mg Gallic Acid Equivalent (GAE) per 100 g of extract[37].

Flavonoid content determination

To assess the total flavonoid content, a measured 20 mg sample of the extract was dissolved in 50 ml of 80% ethanol. Subsequently, 1 ml of this solution was combined with 1 ml of 2% $AlCl_3$ ethanol solution and left to stand for 1 hour. The development of a golden yellow coloration, indicative of flavonoid presence, was quantified at 420 nm with a UV Visible Spectrophotometer (Shimadzu UV1800 Spectrophotometer). The flavonoid concentration was deduced using a Quercetin calibration curve and expressed in terms of mg Quercetin Equivalent (QE) per g of extract [37].

Heavy Metal Screening method

For heavy metal analysis, 1.00 g of the extract was combined with 2 ml of double-distilled water, followed by the addition of 8 ml of a 1:1 nitric acid-perchloric acid mixture and 5 ml of concentrated H_2SO_4. The mixture was heated to 200°C for 30 minutes until a clear solution emanating white fumes was achieved. After cooling, the volume was adjusted to 50 ml with double-distilled water and transferred into a pre-washed PET bottle for subsequent metal analysis[37].

Preparation of Phytosomes

Ipomoea cairica extract phytosomes (IPc-ex-PS) were produced using thin-layer hydration method [38, 39]. In summary, accurately measured amounts of IPc-ex were added to a round-bottom flask and dissolved in 100 mL of a chloroform-ethanol mixture at 60 °C under reflux with magnetic stirring until a uniform suspension was formed. Separately, glyceryl monostearate was dissolved in 25 mL of absolute ethanol and slowly introduced into the solubilized IPc-ex. The mixture was maintained under reflux and stirred at 90 rpm, 60^0C for 2 hours. Subsequently, the solvent was removed using a rotary evaporator under reduced pressure. The resulting sticky, oily, translucent brown substance was further dried under a nitrogen stream, as cryoprotectant, to eliminate any remaining solvent residues [40].

The resulting dry film was then rehydrated in 80 mL of water, and the particle size was reduced by sonicating the suspension in a water bath for 30 minutes.The phytosomes were studied for size, polydispersity, encapsulation effectiveness, zeta potential, surface shape and drug loading as well as in-vitro drug release and stability investigations. To ensure long-term preservation, the phytosomes were freeze-dried at -100ºC using Mannitol as lyo-protectant for approximately 5 days using a ScanvacCoolsafe Touch 100-9 system (Labogene).

Design of Experiment

Response surface design (Box-Behnken design) was utilized to investigateindependent variables effect such as phospholipid content (X1, w:w), temperature (X2,°C) and reflux duration (X3, hr) on particle size and percentage entrapment efficiency (EE) of *Ipomoea cairica* (IPc) extract phytosomes. The 3 independent variables (X1, X2, and X3) were picked at 3 levels, yielding fifteen potential combinations (Table 2). The size of particle and percentage EE of *Ipomoea cairica* extract were used as dependent variables. The experimental trials were run utilizing all fifteen potential combinations of chosen variables [41].

The mathematical model, incorporating interaction terms, coefficient effects, and polynomial components, was evaluated to predict the response using the following equation:

$$Y = b0 + b1X1 + b2X2 + b3X3 + b11X1\ 2 + b22X2\ 2 + b33X3\ 2 + b12X1X2 + b13X1X3 + b23X2X3$$

Here, Y represents the dependent variable, while b denotes the coefficient corresponding to the independent variable X. The primary effects (X1, X2, and X3) indicate the potential overall impact of each factor when varied individually from their low to high levels.The interaction term (X1, X2, and X3) demonstrates how the answer varies when all three parameters are altered at the same time. Polynomial word defines non-linearity. Box-Behnken design was employed to optimize formulation and process parameters, aiming to develop a checkpoint batch with the desired characteristics.

Table 2: Independent variables and responses of Box-Behnken design used in IPc-extract loaded phytosomes (IPc-ex phytosomes) formulation

Independent Variable	**Levels**		
	Level 0	**Level (-1)**	**Level (+1)**
A-Phospholipid concentration	200	100	300
B-Temperature (°C)	40	50	60
C-Reaction time (h)	1	1.5	2
Response	**Desirability constraints**		
Y1-Particle size	Minimum		

Y2-Entrapment Efficiency (%)	Maximize

Evaluation of IPc-ex loadedPhytosomes

The produced *Ipomoea cairica* Phytosomal formulations (IPc-ex phytosomes) were examined for % yield, EE, particle size, in vitro release of drug, zeta potentialand stability. Transmission Electron Microscopy (TEM) was employed to assess the physical characteristics including particle size, zeta potential, size distribution, and encapsulation efficiencyas well as the morphology of phytosomes. The phytosomes were reconstituted in ultrapure water at a 1:10 ratio (phytosomes:water) and subjected to sonication in a water bath for 30 minutes prior to analysis [42].

Entrapment Efficiency

To assess the phytosomal formulation's encapsulation performance, free Quercetin was removed from the emulsion utilizing an Amicon Ultra 100 kDa filter membrane at 14,000×g for 30 minutes. Standard calibration curve of Quercetin was constructed in the previous section for the determination of entrapment efficiency of Quercetin in phytosomes and amount of Quercetin in unknown samples from *in-vitro* dissolution study. The amounts of Quercetin in the filtrate were then measured using the UV-visible technique, as stated previously. The amount of IPc-ex phytosomes equivalent to 20 mg Quercetin was utilized to assess EE. The contents were swirled for 4 hours using a magnetic stirrer before being left to stand for an hour. The clear liquid was decanted and centrifuged at 5000 rpm for 15 minutes. Following centrifugation, the supernatant was filtered using 0.45 µm Whatman filter paper. After appropriate dilution, the absorbance was recorded using a UV spectrophotometer at the corresponding wavelength, and the concentration of Quercetin was quantified. All measurements were conducted in triplicate [43–44].

The EE (%) was determined utilizing the formula:

$EE\ (\%) = T\text{-}S/T \times 100$

Where, T-Total quantity ofQuercetin, S-Quercetin contained in filtrate. In present investigation, vesicle size and % EEwas selected as dependent factors which have potential effect on formulation of phytosomes. The selected parameters were analyzed by ANOVA.

Particle/Vesicle size and Zeta potential determinations

A polydispersity index (PDI) ranging from 0.3 to 0.6 is considered acceptable for drug delivery systems involving lipid-based carriers, like nanoliposome and liposome formulations, as it reflects a relatively homogeneous population of phospholipid vesicles [8]. The particle size diameter and zeta potential were measured at room temperature using a Zeta Potential/Particle Sizer analyzer. The formulations of phytosomes were diluted with water to determine the Zeta potential and particle size [45].

Optimization study

The selected independent variable i.e., phospholipid concentration (mg, Factor A), temperature (^{0}C, Factor B) and reaction time (hr., Factor C) were analyzed by ANOVA and based on the responses, the software has suggested a check point batch which was considered as an optimized batch for further evaluation.The experimental and expected results were compared and a checkpoint batch was selected which was further analyzed for evaluation parameters. The optimized batch thus obtained was further evaluated for various evaluation parameters.

Formulation and Evaluation of IPc-ex phytosomesoptimized batch (IPc-ex-PSopt)

An optimized batch was chosen based on a predetermined goal of minimizing vesicle size and increasing EE. The optimized IPc-exphytosomes batch (IPc-ex-PSopt) was prepared as per formula suggested by design expert. IPc-ex-PSoptwas further evaluated for percentage yield, particle size, EE, TEM, Zeta potential and *in-vitro* release of drug. The dependent responses values were fitted in optimization software and analyzed using ANOVA to obtain an optimized formula.

Transmission Electron Microscopy

The improved IPc-ex-phytosomes' form and aggregation were investigated utilizing a TEM, the JEOL-JEM-1011 (JEOL-Tokyo, Tokyo, Japan). The phytosome sample was suspended in distilled water, and a single drop was placed onto a carbon-coated grid. To enhance contrast, 1% phosphotungstic acid was used as a negative stain. The sample was then allowed to dry at ambient temperature for 15 minutes prior to visualization [46].

***In-vitro*release study of IPc-ex-PSopt**

The dialysis bag technique was used to investigate *in-vitro* drug release from IPc-ex-PSopt [45-46]. The dialysis bag procedure involved introducing pre-weighed samples of IPc-ex-PSopt(equivalent of Quercetin dosage) into 2 pre-soaked dialysis bags. The phytosomes were dispersed in 100 mL of phosphate-buffered saline (PBS) at pH 7.4 and maintained at 37 ± 0.5 °C with continuous stirring at 100 rpm using a magnetic stirrer. Samples were collected at predetermined time intervals (0, 0.5, 1, 2, 4, 6, 8, and 12 hours) and immediately replaced with an equal volume of fresh PBS to maintain sink conditions.The removed samples were tested for Quercetin concentration using UV-visible spectrophotometry. The experiment was performed in triplicate, and the results were reported as mean ± standard deviation (SD).

***In-vitro* Anti-Inflammatory Egg albumin denaturation assay**

Finding substances or agents that might hinder or prevent egg albumin denaturation under particular conditions is the main goal of egg albumin denaturation assay. Proteins lose their biological activity when their structures are altered, a process known as denaturation. The experiment utilises egg albumin as a model protein, which is denaturated through the use of extreme heat, certain pH levels, or other substances that cause protein denaturation. Denaturation causes changes to the physical characteristics and functional inertness of egg albumin by changing its original conformation. To measure the anti-inflammatory effects of a drug or chemical, the egg albumin denaturation assay measures how well it can prevent or lessen the denaturation of egg albumin[47].

Procedure

A 5 mL reaction mixture was prepared by combining 0.2 mL of fresh hen's egg albumin, 2.8 mL of phosphate-buffered saline (PBS, pH 7.4), and varying volumes of extract and IPc-ex-PSopt to achieve final concentrations of 50, 100, 150, and 200 µg/mL.As a control, same amount of double-distilled water was used. After heating at 70°C for 5 minutes, the mixtures were incubated in a BOD incubator at 37±2°C for 15 minutes. When the produced samples cooled, they showed absorbance at 660 nm.The drug that was considered the gold standard was Diclofenac sodium [47]. The following formula was utilized to determine protein denaturation % inhibition:

$$\text{Percentage inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Results and Discussion

In this research, preliminary pharmacognostical evaluations, including morphological and microscopic examinations of *Ipomoea cairica*, were performed. The plant material is authenticated by BSI, Dehradun (Ass No. 1190). Additionally, physicochemical analyses like assessment of moisture content, foreign matter, various extractive values, ash values, and fluorescence characteristicswere carried out to determine the standard parameters of the plant species *Ipomoea cairica*.Further, *Ipomoea cairica* extract was prepared and characterized for various Pharmacognostical and pharmaceutical parameters.

Morphological Characterization of *Ipomoea cairica*

MorphologicallyIpomoea cairicais a climbing woody and herbaceous weed with five-fingered shape like leaves and with funnel like shaped flower that can reach lengths of up to 3-4 feet. In tropical

and subtropical it is used as traditional herbal medicine to treat variant diseases but inMizoram, it is just considered as a normal weed not used for therapeutic properties(12–18). This weed is generally found on the area near about the railway tracks, in fields and dense forestas woody climber (19).

Root: A perennial climber herb or creeper that is firmly ingrained and from which sprouts can emerge is eventually established by tuberous roots, which at first take the shape of a taproot. These roots are brown outside and white inside in appearance.

Leaves: Along the stem, the leaves are alternately oriented and green, palmately lobed, dissected into 5-7 leaflets with very little hair. The measurement of leaves as per length and width, the leaves on petioles range from 2 1/2 to 5 1/2 inches and 100 mm in diameter. There are two nectar-filled glands at the point where the petiole meets the base of the leaf blade and crushed leaves are not aromatic [48].

Stems: In general the stems are 5m long, smooth and can trail along the ground or clamber up other plants. Usually very slightly hairy, twining, and can climbs about a reachability of a height six-one and a half feet long.

Flowers: The flowers of I.p. plant is trumpet-shaped, purple to pinkish in colour, very beautiful and center portion is darker in colour (fig 1A, 1B). The flower closes in mid-afternoon and available in following measurement about 30-60 mm long, 40-60 mm in diameter and area between the leaf petioles and the stem produces single flowers. It blooms throughout the year, and in a population you may find flowers and fruits occurring simultaneously.

Fruit: The fruit capsule is round in shape, 8-12 mm in diameter and surrounded with hairy smooth spikes outside. Usually seeds are green when unripe and dark brown in colour when ripe and mature properly. The seeds of fruit is known as reticulate seed or dimpled seed, which is full of berries (fig 1C).

Seeds: The seeds are oval shaped, *blackish brown in colour and covered with* some sort of hairy spikes/Trichomes. *The thickness of seed covering hairs is about 0.01-0.02 mm* from the attachment margine (fig 1D).

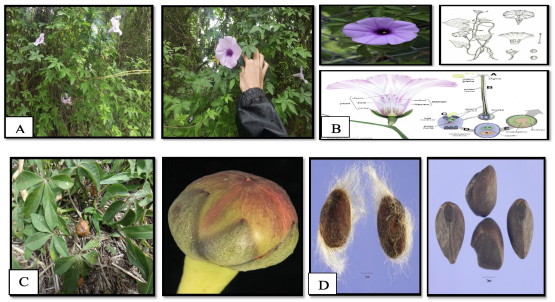

Fig.1: Diagrammatic representation of *Ipomoea cairica*. Morphology of flower (A); Internal parts of flower (B); Structure of *Ipomoea cairica* fruit (C); Structure of *Ipomoea cairica* seeds (D)

Microscopical Examination

Microscopical characterization of fine powderof *Ipomoea cairica* leaves was done and various parameters were studied. Various microscopical characteristics were shown in fig 2. The epidermis was polygonal and parenchymatous. The trichomes were glandular, xylem vessels were reticulate. Stone cells, stomata and pollen grain were also observed in the sample.

Determination of Extractive values

The different extracts of plant *Ipomoea cairica* (IPc-ex) were characterized and following outcomes were found, which were shown in table 3. The extract was dried in a vacuum desiccator to achieve a consistent weight and extractive value. The extractive value report showed that the hydroethanolic extract shows the best results. Thus, crude material of IPc, extracted through hydroethanolic solvent by using soxhlation process was used for further analysis.

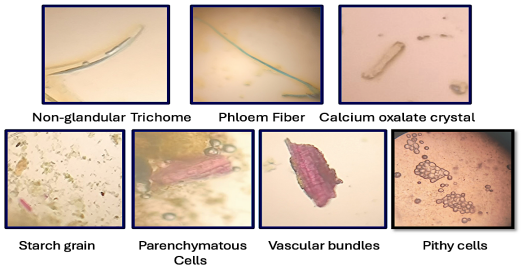

Fig.2: Illustration of Powder Microscopy of *Ipomoea cairica* leaves Powder

Table 3: Characterization of various solvent-based *Ipomoea cairica*leaf extract

Extract	Colour of extract	Odour	Sense of touch	Wt. of plant material (g)	Amt of extract (gm)	%Extractive Value (w/w)
Petroleum ether	Light Yellow	Characteristics	Sticky	15	0.40	2.67
Hydro-ethanol	Light Greenish	Characteristics	Sticky	15	1.26	8.4
Acetone	Dark Green	Characteristics	Sticky	15	0.51	3.4
Ethyl Acetate	Brownish-Green	Characteristics	Sticky	15	0.63	4.2
Chloroform	Green	Characteristics	Sticky	15	0.24	1.6
n-hexane	Dirty	Characteristics	Powder	15	0.20	1.34
	Green					

Ash Value

Ash value is helpful in establishing the purity and authenticity of sample and is a crucial quality criterion [33]. Acid insoluble ash, total ash value and water-soluble ash was represented in table 4. The results are complying with the results reported by Srivastava D. et al., 2014 [49].

Table 4: Ash Content of *Ipomoea cairica* plant

Parameters	% w/w
Total Ash	3.68%
Water Soluble Ash	1.06%
Acid Insoluble Ash	0.88%

Foreign Organic matter analysis

A therapeutic plant material must be completely devoid of any obvious indications ofcontamination from any organic foreign components, including insects, molds andanother animal contaminations [33]. It was found thatno foreign organic matter was found in powdered material of *Ipomoea cairica*.

Fluorescent examination of *Ipomoea cairica*

An effective technique for biochemical analyses and medical diagnostic procedures isfluorescence analysis. High sensitivity fluorescence detection has been achievedusing a variety of detection techniques [34]. The *Ipomoea cairica* plant florescent analysis was briefed in table 5.

Table 5:Fluorescent Analysis of *Ipomoea cairica* Plant

Sample	Day light	Fluorescence light
Powder sample	Dark green	Greenish
Treated with dilute nitric acid	Yellow-Orange	Orange
Treated with sodium hydroxide in water	Brown	Magenta
Treated with hydrochloric acid	Yellowish green	Greenish yellow

Treated with dilute sulphuric acid	Dark Pink	Strong Pale blue
Treated with antimony trichloride	Light brown	Voilet

Loss on Drying or Moisture content

An effective food raw material's moisture content is the primary determinant of howwellthe drying process went. Moisturecontent in sample was determined. The % moisture content was found to be 0.15 %w/win dried leaves and about 87.62% in fresh leaves. Sutapa Chaudhary et al., (2015) reported the moisture content about 89.25% in fresh leaves. Our study showed nearer moisture content [13].

Phytochemical analysis

Various extracts obtained from *Ipomoea cairica* plant were evaluated for the presence of various phytoconstituents occurs in plant (Table 6).

Table 6: Results of phytochemical screening of *Ipomoea cairica* hydroethanolic extract (IPc-ex)

Phytoconstituent	**Method**	**Hydroethanolic extract of *Ipomoea cairica*(IPc-ex)**
Flavonoids	Shinoda Test	+
	Lead acetate Test	+
Alkaloids	Wagner Test	+
	Hager's Test	-
	Dragendroff's test	-
	Mayer's test	-
Tannins and Phenols	Lead acetate test	-
	Bromine water test	+
	Acetic acid solution test	+
	Dil. Potassium permanganate test	-
	Dil. Iodine solution test	+
	Dil. HNO_3 test	-
Cardiac glycoside	Legal test	+
Proteins	Biuret test	+
	Xanthoprotein test	-
	Test for protein containing sulphur	-

TLC Fingerprinting analysis of hydroethanolic extract of *Ipomoea cairica* (IPc-ex) leaves

The Rf value of the hydroethanolic extract of *Ipomoea cairica* (L.) (IPc-ex) was determined and compared with that of standard Quercetin (Table 7, Fig. 3). TLC analysis confirmed the Quercetin presence in the extract, exhibiting Rf value of 0.9. Similar results were shown by Vishwakarma P. et al., 2025[34].

Table 7: TLC analysis of hydroethanolic extract of *Ipomoea cairica*(IPc-ex)

Mobile Phase	**Observed Results**
Toluene: Ethyl acetate (7:3)	Not suitable
Toluene: Ethyl	**Most suitable**

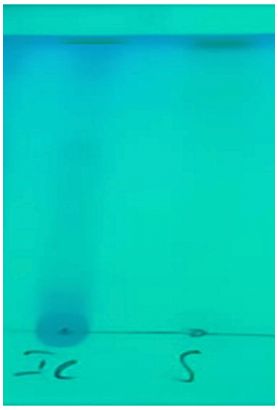

Fig.3: TLC study of *Ipomoea cairica* plant extract (IPc-ex) with standard Quercetin

acetate (6:4)	
Toluene: Ethyl acetate (8:2)	Not suitable
Toluene: Ethyl acetate (5:5)	Not suitable

Distance travelled by Mobile Phase: 5 cm
Spot distance: 4.5
Rf value: 0.9

Characterization of *Ipomoea cairica*extract (IPc-ex)

Ipomoea cairica extract (IPc-ex) was characterized for Quercetin content, TPC and TFC and results were depicted in following section.

Quercetin content

Quercetin content was determined with the help of standard calibration curve of Quercetin prepared for calculating total flavonoid content.

TPCdetermination

The TPC of the IPc hydroethanolic extract was measured using FC method. TPC values were then calculated from the calibration curve y=0.0037x,(R^2=0.9942), where y denotes absorbance and c is Gallic acid solution (mg/mL) concentration represented as mg GAE/g.The TPC of hydroethanolic extract of *Ipomoea cairica*(IPc-ex) was determined and represented in table 6 & fig 6.

Total flavonoid content (TFC)

The TFC of IPc-ex ethanolic extract was calculated utilizing standard Quercetin (QCE). The TFC values were calculated from calibration curve y=0.0119x, R^2=0.9926, where y is absorbance and c is concentration of QCE solution (mg/mL) expressed as mg QCE/g dried extract. The flavonoid content of *Ipomoea cairica* (L.) hydroethanolic extract (IPc-ex) was determined and shown in Table 8 & fig 4.

Ojha S. et al., (2024) investigated the phytochemical composition and anti-oxidant activities of five medicinal plants traditionally used in Indian medicine for the treatment of inflammation, cancer and oxidative stress-related conditions and reported the TPC and TFC value of *Ipomoea cairica* extract. In another study, conducted by Banerjee D. et al., (2013), total phenolic content of different extracts of *Ipomoea-per-caprae* was reported. The obtained values in the present work are complying with the reported values[50-51].

Table 8: TPC, TFCand IC50 values of different extracts of *Ipomoea cairica*(IPc-ex)

Extract of *Ipomoea cairica* (L.) (IPc-ex)	**TFC (mg QCE/g)**	**TPC (mg GAE/g)**	**IC50 Value(µg/ml)**
Hydroethanolic extract	26.39±1.56	43.15±1.28	140.21 µg/ml
Ethyl acetate extract	28.73±1.69	95.12±1.11	255.15 µg/ml
Chloroform	15.39±1.77	78.81±1.25	391.62 µg/ml
Acetone	13.64±1.56	57.22±1.19	422.71µg/ml
Petroleum ether	11.58±1.52	36.19±1.18	476.26 µg/ml
n-Hexane	9.87±1.16	25.33±1.36	498.17 µg/ml

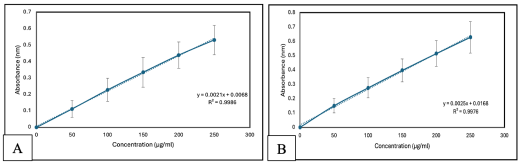

Fig.4: Calibration curve of Gallic acid (A) and Calibration curve of Quercetin (B) for determination of TPC and TFC of hydroethanolic extract of *Ipomoea*

***cairica*(IPc-ex)(mean±SD)**

Total phenolic content and total flavonoid content of different extracts of *Ipomoeacairica* extract (IPc-ex)was determined. Total phenolic and flavonoid contents ofethyl acetate was found to be95.12±1.11mg GAE/g &28.73±1.69 mg QCE/g,which is the maximum value. Total phenolic and flavonoid contents ofhydroethanolic extract was found to be43.15±1.28 mg GAE/g &26.39±1.56 mgQCE/g. It was reported thatthe hydroethanolic extract of *Ipomoea cairica leaves* showed strong anti-inflammatory effects in various experimental models, such as the formalin-induced inflammatory phase test in mice, exhibiting dose-dependent reduction of inflammation [49].Thus, hydroethanolic extract was selected for formulation of phytosomes.

Quercetin Content determination

Quercetin was estimated by using standard calibration curve of Quercetin (Fig. 6) and its content was found to be 1.32 mg/ml in the hydroethanolic extract of *Ipomoea cairica* (IPc-ex). Viswanath A et al., (2016) isolated and characterized Quercetin and our results reported the nearer concentration of Quercetin in hydroethanolic extract of *Ipomoea cairica* [52].Quercetin shows measurable anti-inflammatory activity by downregulating NF-κB/MAPK signaling and reducing mediators such as COX-2, iNOS, TNF-α, IL-6, and IL-1β, with supportive evidence across cell, animal, and human studies including meta-analyses of randomized trials showing C-reactive protein reduction at doses ≥500 mg/day. It also inhibits NLRP3 inflammasome activation in multiple models, linking antioxidant actions to suppression of IL-1β/IL-18 maturation and downstream inflammation [53]. Thus, Quercetin was quantified in the extract of *Ipomoea cairica* (IPc-ex).

Heavy Metal Screening

Ipomoea cairica leaves have been reported to contain lead (2.568 µg/ml), zinc (1.411) µg/ml, chromium (0.614 µg/ml), arsenic 0.755 (µg/ml) and cadmium (0.003 µg/ml) in leaf sample. Similar results were reported by Inala E.R (2024) i.e., lead 2.872 µg/ml, zinc 1.429 µg/ml, chromium 0.763 µg/ml, arsenic 0.846 µg/ml, and cadmium 0.003 µg/ml in the acid digest of leaf samples, while mercury was not detected in that dataset. The study stated the obtained values were within WHO/FAO permissible thresholds for plants, noting that site conditions influence accumulation levels [54]. Our study also reported the nearer values.

Preparation of hydroethanolic extract loaded phytosomes of *Ipomoea cairica* (IPc-ex-PS)

Different factors, like phospholipid concentration, process variables like temperature and time, etc., significantly influence the phytosomes development. For the current study, glyceryl monostearate was chosen as the phospholipid since it has already been extensively used in the development of sustained release delivery methods. The phytosomes were created utilizing the thin-layer hydration approach.Glyceryl monostearate was solubilized in ethanol and slowly added to the solubilized plant extract under reflux and stirring. The optimized phytosomes formulation was further characterized for parameters like size, zeta potential, morphology, *in-vitro* release etc. Glyceryl monostearate helped form the lipid bilayer structure of the phytosomes, encapsulating the plant extract components.

Model Fitting for phytosomes of *Ipomoea cairica hydroethanolic extract* (IPc-ex-PS)

The Box-Behnken design offered fifteen tests under various conditions for specified variables. Table 7 shows the results (particle size and EE) from the various tests.Results suggested that particle size (Y1) of prepared batches ranged between 220.3 to 469.7nm for each batch. In the present study, entrapment efficiency of Quercetin was taken into consideration. It is reported either total phenolic content (TPC) or total flavonoid content (TFC) can

be used as a standard for determination of entrapment efficiency if the entrapped actives are mixed polyphenol fraction or flavonoid content if flavonoid dominates[55-56]. In the present study, the TPC and TFC values for hydroethanolic extract of *Ipomoea cairica* showed 43.15±1.28 mg Gallic acid equivalent/g and flavonoid content was 26.39±1.56 mg Quercetin equivalent/g. Results represented that total phenolic content is more than total flavonoid content in the hydroethanolic extract of *Ipomoea cairica* (IPc-ex). However, Quercetin has shownsuperior antioxidant and anti-inflammatory capacity than other flavonoids [56-58]. Thus, Quercetin was selected as potential phytoconstituent and entrapment efficiency of Quercetin was determined. The Quercetin % EE (Y2) was found to be between 30.83–84.38%. As a result, the data were analyzed using Design-Expert software, which generated a quadratic polynomial equation to confirm the influence of independent variables on the various responses. Furthermore, the magnitude of each factor's effect on the dependent variables was predicted by formulating a separate polynomial equation for each response (Equations 1–2).

$$Y1=437.20+4.79A-93.66B+0.00C+27.73AB-74.50AC+22.60BC-30.24A^2-35.14B^2-35.71C^2 Eq....1$$

$$Y2=75.39+4.02A+14.33B+8.90C-0.542AB+3.71AC-3.77BC-1.85A^2-12.31B^2-8.74C^2 Eq....2$$

Furthermore, analysis of variance (ANOVA) was performed on obtained data to determine significance of results. The model was significant ($p<0.001$) for both answers and accurately predicted the experimental conditions. Furthermore, the low fit value ($p<0.05$) supported the model's appropriateness. Furthermore, the software shown that the coefficient of determination (R^2) for both components is close to one ($R^2>0.9$), confirming that dependent and independent variables are appropriately related [59]. Additionally, the software demonstrated adequate accuracy for the responses (Y1 = 7.638 and Y2 = 18.95), which reflects the signal-to-noise ratioa key indicator of the reliability and precision within the predicted response range. A ratio greater than 4 for all responses indicates a strong and adequate signal, confirming that the developed model is appropriate for navigating the design space [60]. The Design-Expert software also generated 3D surface plots (Table 9, Fig 5) to illustrate the impact of different independent variables on the selected responses. These plots are particularly valuable for visualizing how variations in the level of one factor influence the response in relation to other factors. Since these graphs can display the interaction of only two or more variables at a time, at least one independent variable must be held constant during the analysis [60].

Table 9: Several runs under varied experimental conditions

Formulation blend	Independent variables			Responses	
	Glutaraldehyde (A)	Temperature (B)	Reflux time (C)	Particle size (nm)	%EE
F1	100	60	1.5	120.3	74.82
F2	100	50	2	341.8	66.74
F3	300	60	1.5	229.4	79.58
F4	200	60	2	219.8	70.29
F5	100	50	1	235.2	52.63
F6	200	60	1	132.2	63.75
F7	100	40	1.5	369.7	41.78
F8	300	40	1.5	367.9	48.71
F9	200	50	1.5	335.4	74.37

F10	300	50	1	349.7	55.43
F11	200	40	2	355.3	52.46
F12	300	50	2	158.3	84.38
F13	200	50	1.5	339.5	76.45
F14	200	40	1	358.1	30.83
F15	200	50	1.5	336.7	75.35

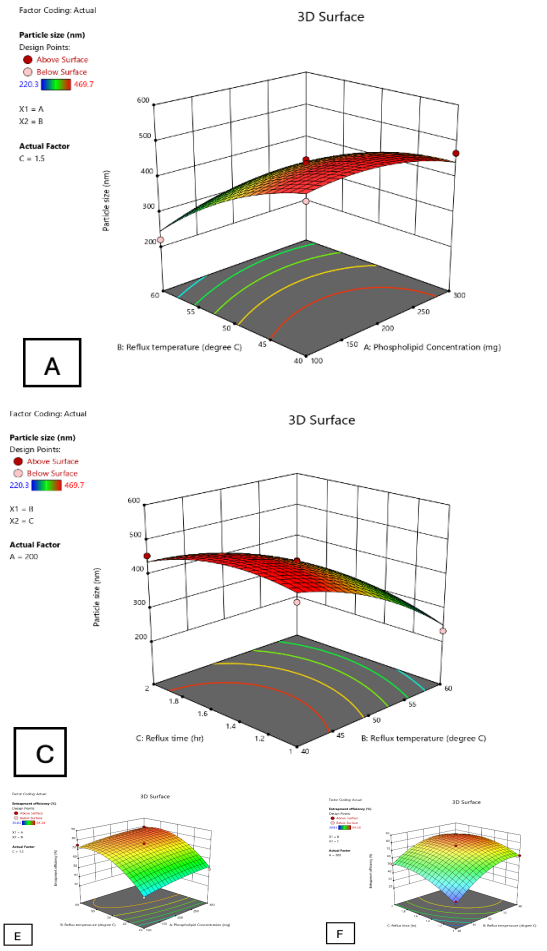

Fig.5: 3D graph showing effects of Phospholipid concentration (A), temperature (B) and reflux time (C) on various responses: (a) particle size and (b) % EE

Optimization and Validation of Development Model

The experimental conditions for formation of IPc phytosomes were refined utilizing a numerical optimization method. This technique proposed alternative alternatives and optimum circumstances for the production of IPc phytosomes. The conditions were chosen based on parameters such as minimal particle size and maximum EE. Design-expert software projected a phospholipid content of 300 mg, a reflux temperature of 600, and a reflux time of approximately 2 hours for the formation of IPc-ex loaded phytosomes (IPc-ex-PSopt). Furthermore, experiments were carried out under the predicted optimal conditions to validate the optimization results. The observed response values for the dependent variables were found to fall within the 95% confidence interval, thereby confirming the reliability and accuracy of the developed model for preparation of IPc-ex-PSopt.

Evaluation of Optimized Formulation

The produced phytosomes of IPc extract under optimal conditions were assessed on a variety of characteristics. The particle size of IPc-ex-PSopt was found to be 118.0±0.25 nm (Fig 6) and the Quercetin% EE wasanalyzed to be79.59±0.21%. The EE and particle size of optimized batch was nearer to the predicted solution given by factorial design, and it confirmed the validity of statistical tool in optimizing formulation and process parameters. The zeta potential of IPc-ex-PSopt was found to be -25.6 mv, within the standard range of -30 to +30, confirmed the stability of prepared phytosomes.

FTIR provides molecular interaction evidence (chemical signature of complexation), whereas TEM establishes the nanoscale architecture (vesicle integrity and morphology), giving orthogonal confirmation of successful phytosome formation and quality[61].This combination links composition–interaction (FTIR) to structure–function (TEM), supporting claims of enhanced stability and delivery performance expected from properly formed phytosomes [62]. FTIR confirms

drug–phospholipid complexation in phytosomes by tracking diagnostic band shifts (e.g., P=O, C=O, P–O–C, and phenolic O–H)[61].FTIR identifies characteristic phosphatidylcholine bands—C=O stretching near ~1735 cm^{-1}, P=O near ~1249 cm^{-1}, P–O–C near ~1057–1093 cm^{-1}, and the choline band near ~970–985 cm^{-1}—serving as anchors to detect interaction-induced shifts in the phytosome versus the individual components and physical mixtures.Interaction and complexation are evidenced by shifts/broadening in extract O–H bands (~3400 cm^{-1}) and changes in phospholipid P=O and C=O bands, indicating hydrogen bonding or other intermolecular interactions consistent with phytosome formation [63].

The optimized formulation (IPc-ex-PSopt) was then tested for compatibility with the extract and excipients using FTIR. The FTIR spectra of IPc leaf extract revealed alcohol (OH), phenol Stretch (3851 cm^{-1}), N-H amine (3325 cm^{-1}), C-H alkane (2923 cm^{-1}), carbonyl compound (C=O)-1728 cm^{-1}, alkene (C=C)-1640 cm^{-1}, methylene group (CH2)-1453 cm^{-1}, and alkynes (C≡H)-1373 cm^{-1} (Fig 7(a)).The physical mixture spectra for extract, phospholipid, and span 80 showed absorption bands at 3853 cm^{-1} (-OH vibration stretching of alcoholic and phenolic compounds), 3307 cm^{-1} (N-H) amine, 1727 cm^{-1} (C=O) carbonyl compounds, 1464 cm^{-1} (CH2), and 1052 cm^{-1} (C-O) ether stretch (Fig 7(b)).The optimized formulation's FTIR spectra retained the drug's distinctive peaks, including O-H vibration (3852 cm^{-1}), primary amine (3391 cm^{-1}), and C-H alkane stretch at 1923 cm^{-1}, indicating compatibility with the selected excipients (Fig 7(c)). Furthermore, the absence of additional peaks and the lack of significant shifts in existing peaks confirmed the successful incorporation of the extract into the phytosomes [64].

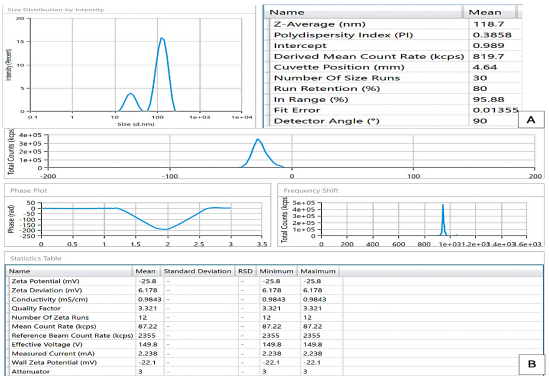

Fig.6: A. Particle size of IPc-ex-PSopt; B. Zeta potential of IPc-ex-PSopt

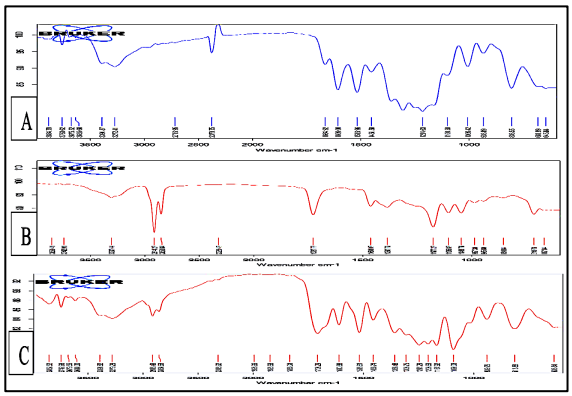

Fig.7: FTIR spectra of Quercetin (pure) (A), FTIR spectra of formulation blend (B), FTIR spectra of optimized formulation (C)

TEM analysis

Most phytosomes are composed of amphiphilic molecules known as phospholipids, which consist of two neutral hydrophobic tails and a positively charged hydrophilic headgroup. Additionally, the solubility of quercetin was enhanced when it was complexed with phospholipids. TEM verifies the vesicle architecture by directly visualizing spherical morphology, lamellarity, and size to ensure proper nanoscale assembly and stability. TEM provides direct visualization of vesicle morphology (typically spherical), lamellarity, and aggregation state, confirming that complexes self-assemble into nano or submicron vesicles rather than amorphous aggregates [65]. TEM provides direct visualization of vesicle morphology (typically spherical),

lamellarity, and aggregation state, confirming that complexes self-assemble into nano- or submicron vesicles rather than amorphous aggregates. TEM analysis revealed that the optimized phytosome formulation, IPc-ex-PSopt, exhibited a more spherical morphology with a particle size of approximately 119 nmclosely aligning with the 118 nm size measured using the zeta sizer (Fig 8).

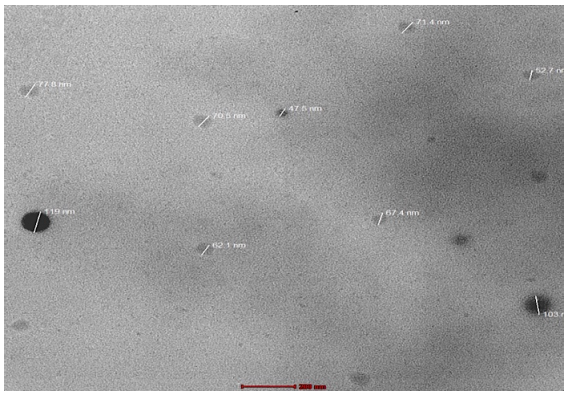

Fig.8: TEM image of IPc-ex-PSopt

***In-vitro* drug release study**

The *in-vitro* release of IPc-ex-PSopt for Quercetin was investigated. The findings showed that approximately 70% of plant's active constituent was permeated in 8 hours (Fig 9). The phytoconstituent release was estimated by U.V. visible spectrophotometer. These findings suggest that produced phytosomes can effectively increase the anti-inflammatory efficacy of IPc-ex-PSopt, making them appropriate for the creation of an IPc-based sustained drug delivery system. Fig 9 displayed the findings of an *in-vitro* diffusion research. The % release of drug was found to be 87.75±1.27% at the end of 12 hr.

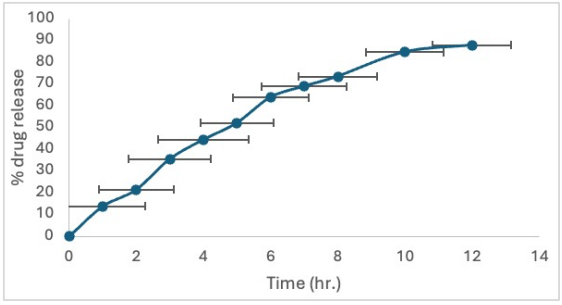

Fig.9: *In-vitro* drug diffusion data of IPc-ex-PSopt

Evaluation of *in-vitro* anti-inflammatory effect of IPc-ex-PSopt

The anti-inflammatory effectiveness of IPc-ex andIPc-ex-PSopt formulation was evaluated by egg albumin denaturation assay and compared to standard anti-inflammatory agent, Diclofenac sodium. Diclofenac sodium had an inhibition rate of 85.25±0.62% at a dose of 100 µg/mL.The IPc-ex showed the inhibition rate of 79.11±0.73%, while, the optimized formulation exhibited a concentration-dependent inhibitory effect, with a maximum inhibition of 87.49±0.79% at 200µg/mL and 37.61±0.75% at 50 µg/mL. While Diclofenac sodium displayed greater efficacy and functioned as the reference standard, the optimized formulation showed significant anti-inflammatory properties (Fig 10).

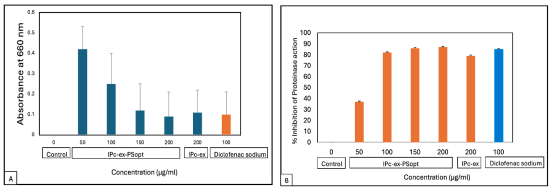

Fig. 10: Protein denaturation study of IPc-ex and IPc-ex-PSopt formulation. A. Absorbance of samples & Standard drug at 660 nm; B. % inhibition of Proteinase action

Discussion

Ipomoea cairica (L.) Sweet, commonly called railway creeper or purple morning glory, is a vigorous perennial vine from the Convolvulaceae family widely distributed in tropical and warmer temperate climates. Traditionally utilized in folk medicine, particularly in Brazil for rheumatism, inflammation, and other ailments, its extracts have drawn scientific interest for bioactive compounds like caffeoylquinic acids and flavonoids. This plant's phytochemistry supports potential in anti-inflammatory formulations, aligning with ongoing research into natural therapeutics [14]. Ethanolic extracts exhibit dose-dependent antinociceptive and anti-inflammatory effects, reducing inflammatory

responses in models like the formalin test, possibly via inhibition of histamine release by caffeoylquinic derivatives [14].

The present study illustrates that a hydroethanolic leaf extract of *Ipomoea cairica* can be effectively encapsulated into phytosomes optimized using the Box–Behnken Design (BBD). This process results in an optimized nanosystem with an average size of approximately 118 nm, a zeta potential of −25.6 mV, a Quercetin entrapment efficiency of about 79.6%, a 12-hour cumulative release of about 87.8% and a concentration-dependent *in-vitro* anti-inflammatory activity (egg albumin denaturation) that slightly surpasses Diclofenac at higher extract-equivalent concentrations. This substantiates the formulation's functional potential while aligning with the extract's known pharmacological properties. The choice of Quercetin as the marker for entrapment efficiency is consistent with the phytochemical profile quantified in the extract and Quercetin's established anti-inflammatory mechanisms, enhancing the interpretability of loading metrics in relation to biological effects. The observed inhibition in the protein denaturation assay complements previous *in-vivo* findings, where *Ipomoea cairica* ethanolic extract reduced the inflammatory phase of the mouse formalin test in a dose-dependent manner, thereby linking classical pharmacology with delivery-enabled enhancement of activity *in-vitro* [66].

The nanoarchitectural and interaction evidence was orthogonally validated by TEM and FTIR, respectively. TEM revealed spherical vesicles approximately 119 nm in size, aligning with DLS findings. Concurrently, FTIR maintained the diagnostic bands of the extract and lipid, featuring interaction-consistent characteristics, collectively indicating successful phyto–phospholipid complexation and coherent vesicle assembly. These results reflect best practices for phytosome characterization, where FTIR identifies hydrogen-bonding/complexation signatures at the O–H, C=O, and P=O domains, and TEM visualizes vesicle morphology and lamellarity, corroborating the structural integrity that underpins stability and delivery performance [21].

The 12-hour release reaching ~88% supports a sustained, diffusion-governed release compatible with improved exposure of phenolics/flavonoids, consistent with phytosome platforms known to enhance permeability, protect actives, and improve pharmacokinetics for polyphenolic agents. Statistically, the BBD-derived quadratic models for particle size and quercetin entrapment were significant ($p<0.001$) with high R^2 and adequate signal metrics, allowing robust prediction within the design space and identification of favorable operating windows for lipid content, temperature, and time. The methodological choice of BBD aligns with contemporary phytosome optimization literature across botanicals, where three-level designs efficiently capture main, quadratic, and interaction effects while avoiding extreme corner points and limiting the experimental burden. The optimized response profile, with a size of ~118 nm and EE of ~79.6%, fell within the range commonly reported for flavonol phytosomes. However, Quercetin-loaded phytosomes can achieve smaller sizes and higher entrapment when using purified actives and tuned phospholipid ratios, highlighting realistic trade-offs when encapsulating chemically complex extracts rather than single molecules [38].

The hydroethanolic extract's TPC (~43 mg GAE/g) and TFC (~26 mg QE/g) reveal a notable presence of phenolics and flavonoids within a biocompatible solvent system, which complements the extract's anti-inflammatory potential and the strategic enhancement of delivery through phospholipid complexation. This is in line with previous pharmacognostic studies and traditional medicinal uses, where the anti-nociceptive and anti-inflammatory properties of Ipomoea species have been linked to compounds like caffeoylquinic acids and flavonols, including quercetin. This connection supports a credible mechanistic link between the quantified chemistry, marker selection, and the observed bioactivity in the current formulation. Importantly, this manuscript covers heavy metal

screening and basic quality attributes (ash values, fluorescence, foreign matter, and LOD), which are vital for advancing toward standardized botanicals, especially in light of environmental variability and the necessity for safety dossiers in food or biomedical contexts [66].

When compared, the entrapment efficiency and nanoscale control of the phytosomes in this study align with those of BBD-optimized phytosomes reported for other plant matrices and purified flavonoids. However, purified Quercetin systems can achieve EE >90% and sizes ~60–90 nm with optimized lipid ratios and cholesterol modulation, indicating potential for further refinement if a more Quercetin-focused specification is desired (e.g., lipid choice, lipid: extract ratio, and cholesterol content). The *in-vitro* anti-inflammatory assay (protein denaturation) serves as a practical screening tool. Future research could explore cell-based cytokine modulation or enzyme-target assays (e.g., COX-2/iNOS/NF-κB) and *in-vivo* inflammatory models, where existing Ipomoea cairica data provide a solid historical baseline to evaluate whether the phytosome formulation offers a tangible pharmacodynamic advantage over the crude extract. Lastly, the novelty lies in the incorporation of a hydroethanolic *Ipomoea cairica* leaf extract into phytosomes optimized by BBD, showcasing enhanced *in-vitro* anti-inflammatory activity. This is supported by the lack of previous *Ipomoea cairica*phytosome reports and aligns with broader phytosome literature that validates this platform for polyphenolic actives [67].

Conclusion

The study successfully designed and optimized *Ipomoea cairica* leaf extract-loaded phytosomes using a Box–Behnken framework, achieving nanoscale vesicles with confirmed drug–phospholipid complexation and measurable *in-vitro* anti-inflammatory activity that outperformed the crude extract within the tested conditions. These findings demonstrated that a rational delivery system can enhance the functional expression of a hydroethanolic botanical rich in phenolics/flavonoids, aligning chemistry, process, and bioactivity into a coherent, translational package. Optimization via Box–Behnken Design identified a formulation and process window that balanced particle size, entrapment efficiency and dispersion quality, enabling predictable, high-quality nanosystems from a complex plant matrix. FTIR and TEM provided orthogonal confirmation of phyto–phospholipid complexation and spherical vesicle morphology, corroborating that the intended nanoscale architecture was achieved and is consistent with sustained release behavior observed *in-vitro*. Marker-based quantification linked entrapment and release to a pharmacologically relevant constituent, while anti-inflammatory assays indicated formulation-dependent enhancement versus the crude extract, supporting the delivery rationale. This work is among the first to couple systematic response-surface optimization with extract-loaded phytosomes for *Ipomoea cairica*, moving beyond qualitative reports to a quantitative, design-space definition for a standardized nanoformulation. The study integrates compositional readouts (e.g., phenolic/flavonoid burden and a targeted marker) with structure–function evidence (FTIR/TEM and release), creating a traceable chain from chemistry through carrier architecture to bioactivity that is uncommon in prior *Ipomoea* literature. By anchoring entrapment efficiency to a specific analyte and validating vesicle formation with complementary methods, the formulation advances from descriptive encapsulation to a reproducible, specification-ready platform. The optimized phytosome can serve as a standardized intermediate for anti-inflammatory product development, offering improved handling, stability potential and dose uniformity relative to crude extracts for research and preformulation pipelines. The defined process parameters and acceptance attributes enable reproducible scale-out in laboratory settings and inform scale-up risk assessments,

shortening development cycles for botanically-derived prototypes. In sum, a design-of-experiments-guided phytosome strategy transformed a hydroethanolic extract of *Ipomoea cairica* into a nanoscale, interaction-verified carrier that enhanced *in-vitro* anti-inflammatory performance and established clear, testable quality attributes across composition, structure and function. These outcomes position the formulation as a credible foundation for next-stage mechanistic validation, stability studies and application-focused development in anti-inflammatory nutraceutical or topical biomedical contexts.

References

1. Alamgir ANM. Medicinal, non-medicinal, biopesticides, color-and dye-yielding plants; secondary metabolites and drug principles; significance of medicinal plants; use of medicinal plants in the systems of traditional and complementary and alternative medicines (CAMs). Therapeutic use of medicinal plants and their extracts. 2017; 1: 61-104.
2. Stewart D, et al. Inflammation, immunity and allergy. Anaesthesia & Intensive Care Medicine. 2021; *22*(8): 488-493.
3. Ali RA, et al.Cardiovascular diseases and Rheumatology. Skills in Rheumatology. 2021; 353-381.
4. Yatoo MI, et al. Anti-inflammatory drugs and herbs with special emphasis on herbal medicines for countering inflammatory diseases and disorders-A review. Recent patents on inflammation & allergy drug discovery. 2018; *12* (1): 39-58.
5. Yasmeen F, et al. Understanding Autoimmunity: Mechanisms, Predisposing Factors, and Cytokine Therapies. International Journal of Molecular Sciences. 2024; 25 (14): 7666. https://doi.org/10.3390/ijms25147666.
6. Li X, et al. Multifunctional nanoparticle-mediated combining therapy for human diseases. Sig Transduct Target Ther.2024; 9: 1. https://doi.org/10.1038/s41392-023-01668-1.
7. Jin J, et al. Factors affecting therapeutic compliance: A review from the patient's perspective. Ther Clin Risk Manag. 2008; 4(1): 269-86. doi: 10.2147/tcrm.s1458.
8. Radu, AF, et al. Management of rheumatoid arthritis: an overview. Cells. 2021; 10 (11): 2857.
9. Kamboj, VP. Herbal medicine. Current science. 2000; *78*(1): 35-39.
10. Theofilou, VI, et al. Immunologic diseases. Burket's Oral Medicine. 2021; 705-743.
11. Sudhendu M, et al.Studies on *Ipomoea cairica (L.)* sweet-A promising Ethnomedicinally important plant. JIPBS. 2015; 2 (4): 378-395.
12. Ilesanmi OB,et al.Hydromethanolic extract of Ipomoea cairica leaf extract ameliorate the oxidative activity of cadmium chloride in the hearts and testes of male Wistar rats. Al-Bahir Journal for Engineering and Pure Sciences. 2023; 3 (1):34-40.
13. Choudhury SC,et al. Studies on *Ipomoea Cairica* (L.) Sweet - A Promising Ethnomedicinally Important Plant. Journal of Innovations in Pharmaceutical and Biological Sciences. 2015; 2 (4): 378-95.
14. Ferreira AA, et al. Antinociceptive effect from Ipomoea cairica extract. J Ethnopharmacol. 2006; 105 (1-2): 148-53. doi: 10.1016/j.jep.2005.10.012.
15. Rojas-Sandoval J, *Ipomoea cairica* (five-fingered morning glory)CABI Compendium, doi:10.1079/cabicompendium.28786, CABI, 2022.
16. Gumula I, et al. Antibacterial Properties of Phytochemicals Isolated from Leaves of *Alstoniaboonei* and Aerial Parts of *Ipomoea cairica*. Natural Product Communications. 2024;19(9). doi:10.1177/1934578X241286425.
17. Vanlalhruaii R. Evaluation of phytochemical contents of *Ipomoea cairica* (L) sweet-a qualitative approach. Science Vision. 2014; 14 (3): 145-151.
18. Álvarez VV, et al. Bioguided Phytochemical Study of *Ipomoea cairica* Extracts with Larvicidal Activity against *Aedes aegypti*. Molecules. 2022;27(4):1348. doi: 10.3390/molecules27041348.

19. Deepa S, et al. *Ipomoea cairica*: A medicinal weed with promising health benefits. International Journal of Information Research and Review. 2015; 2 (05): 687-694.
20. Ralte V, et al. Evaluation of phytochemical contents of *Ipomoea cairica* (L) Sweet–a qualitative approach. 2014. Science Vision; 14 (3): 145-151.
21. P K, et al. Phytosome Technology: A Novel Breakthrough for the Health Challenges. Cureus. 2024;16(8):e68180. doi: 10.7759/cureus.68180.
22. Chen RP, et al. Phytochemical Delivery Through Transferosome (Phytosome): An Advanced Transdermal Drug Delivery for Complementary Medicines. Front. Pharmacol. 2022; 13:850862. doi: 10.3389/fphar.2022.850862.
23. Alharbi WS, et al. Phytosomes as an Emerging Nanotechnology Platform for the Topical Delivery of Bioactive Phytochemicals. Pharmaceutics. 2021;13(9):1475.
24. Patel PM, et al. Phytosomes: An Emerging Technique for improving herbal drug delivery. J Phytopharmacol. 2023; 12 (1): 51-58. doi:10.31254/phyto.2023.12108.
25. Abd-El-Aziz NM, et al. Application of Box-Behnken design for optimization of phenolics extraction from *Leontodon hispidulus* in relation to its antioxidant, anti-inflammatory and cytotoxic activities. SciRep. 2022;12:8829. https://doi.org/10.1038/s41598-022-12642-2.
26. Venkatachari KM, et al. Box -Behnken Design Approach to Method Development for Irinotecan Hydrochloride Trihydrate by Using RP-HPLC Method. Biomed &Pharmacol J. 2025;18(1): 617-624.
27. Sriram Pavani, et al. Use of Box-Behnken Experimental design for optimization of process Variables in Iontophoretic delivery of Repaglinide. J Young Pharm. 2016; 8 (4): 350-355.
28. Omji Porwal. Box-Behnken Design-based formulation optimization and characterization of spray dried rutin loaded nanosuspension: State of the art, South African Journal of Botany. 2022; 149: 807-815.
29. Martins, MS, et al. Anatomy of stem and root of *Ipomoea cairica* (L.) Sweet (Convolvulaceae)-the Lianescent Vascular Syndrome. Balduinia, 2021; (67): 10-16.
30. Rishabh K, et al. Optimized extraction technique and solvent to enhance the recovery of bioactive polyphenols from Viola canescens wall. Ex Roxb. A case study using OFAT and chemical characterization using UPLC-PDA, UHPLC-Q-TOF-IMS and GC–MS. Microchemical Journal, 2025; 212: 113170.
31. Rodríguez De Luna SL, et al. Environmentally Friendly Methods for Flavonoid Extraction from Plant Material: Impact of Their Operating Conditions on Yield and Antioxidant Properties. ScientificWorldJournal.2020:6792069. doi: 10.1155/2020/6792069.
32. Deleanu M, et al. Formulation of Phytosomes with Extracts of Ginger Rhizomes and Rosehips with Improved Bioavailability, Antioxidant and Anti-Inflammatory Effects *In-Vivo*. Pharmaceutics, 2023; 15(4), 1066-72.https://doi.org/10.3390/pharmaceutics15041066.
33. John GN, et al. Phytochemical and proximate analysis of *Ipomoea cairica* tuber. Global Journal of Pure and Applied Sciences, 2021; *27*(1): 11-16.
34. Vishwakarma P, et al. Extraction phytochemical analysis and isolation of compound from *ipomoea cairica* root extract. Cuestiones de Fisioterapia, 2025; *54*(4): 4966-4978.
35. Daniel, C. Phytochemical and proximate analysis of *Ipomoea cairica* tuber. Global Journal of Pure & Applied Sciences. 2021; *27*(1).
36. Srivastava N, et al. Development and Validation of UV Spectrophotometric Method for the Quantitative Estimation of Quercetin in Bulk Followed by its Solubility Studies. J Appl Spectrosc.2024; 91:700–708. https://doi.org/10.1007/s10812-024-01773-1.
37. Otabil J, et al. Hydroethanolic Leaves Extract of Amaranthus Cruentus Protects Against Lead-Induced Hepatorenal Toxicity in Rats. Biomed. Res. Ther. 2024; 11(5):6457-6473.

38. Rathee S, et al. Optimization and development of antidiabetic phytosomes by the Box–Behnken design. Journal of liposome research. 2018; *28*(2): 161-172.
39. Angreni A, et al. Development of Phytosomes-Black Tea extract complex by different methods and study of Cholesterol's effect on Entrapment efficiency. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2017; 8 (1): 995-1021.
40. Jaiswal AN, et al. Cryopreservation: A Review Article. Cureus. 2022;14(11):e31564. doi: 10.7759/cureus.31564.
41. Singh A, et al. Development and optimization of Andrographis paniculata extract-loaded phytosomes using Box-Behnken design approach. Journal of Integrated Science and Technology. 2023; 11(4): 558-558.
42. Das MK, et al. Design and evaluation of phyto-phospholipid complexes (phytosomes) of rutin for transdermal application. Journal of Applied Pharmaceutical Science. 2014; 4(10): 051-7.
43. Danaei M, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. Pharmaceutics. 2018; 10(2): 57.
44. Hindarto CK, et al. Effect of mole ratio on physicochemical properties of luteolin-loaded phytosome. The Pharma Innovation Journal. 2017; 6(12): 96-101.
45. Alfaifi MY,et al. Anti-tumor effect of PEG-coated PLGA nanoparticles of febuxostat on A549 non-small cell lung cancer cells. Biotech. 2020; 10: 133.
46. Zheng Y, et al.mPEG-icariin nanoparticles for treating myocardial ischaemia. Artif. Cells Nanomed. Biotechnol. 2019; 47: 799–809.
47. Monitoring Protein Denaturation of Egg White Using Passive Microwave Radiometry (MWR) [Internet]. Available from: https://www.mdpi.com/2075-4418/12/6/1498.
48. http://www.consultaplantas.com/index.php/en/plants-from-d-to-l/2271-*ipomoea-cairica*-or-coast-morning-glory-care-and-growing.
49. Deepa Srivastavaet al.Morphological and Physicochemical Analysis of Ipomoea cairica Leaves. Asian J. of Adv. Basic Sci.2014; 3(1): 222-22.
50. Sunil K Ojha, et al. Design of an Iterative Model Integrating Bacterial Foraging Optimizer and Q-Learning for Enhanced Congestion Management in Wireless Networks. Frontiers in Health Informatics. 2024; 13 (3): 1426-1438.
51. Banerjee D, et al. Antioxidant and Antiinflammatory Activities of Different Solvent Extracts and Isolated Compounds of Ipomoea pes-caprae (L) Sweet of Sunderban Mangrove Eco-complex. Asian Journal of Chemistry. 2025; 25 (9): 4997-5000.
52. Viswanath A, et al. Isolation, Characterization and Estimation of Quercetin from Ipomoea sepiaria Koenig Ex. Roxb by UV Spectrophotometry. Inventi RapidPharm Analysis & Quality Assurance. 2016; 1:1-8.
53. Aghababaei F, et al. Recent Advances in Potential Health Benefits of Quercetin. Pharmaceuticals (Basel). 2023;16(7):1020. doi: 10.3390/ph16071020.
54. Eneni RI.Assessment of Heavy Metals in selected Medicinal Plants in Bayelsa State, Nigeria. Nigerian Journal of Pharmaceutical and Applied Science Research. 2024; 13 (3): 18-21.
55. Sucilawaty R, et al. Development of Kopasanda Leaves Extract (Chromolaena odorata (L.)) Phytosome using solvent evaporation method. IJDDT. 2024; 14 (3): 1586-1590.
56. Thi-Thu-Giang Vu, et al.Preoparation and physicochemical evaluation of hydrogel containing Quercetin phytosomes. Pharm Sci Asia. 2021; 48 (2): 122-138.
57. Ay M, et al. Quercetin. In: Nutraceuticals. Elsevier. 2016: 447-52.
58. Watson R. Complementary and alternative therapies and the aging population. Elsevier, 2009.
59. Nanda A, et al. Design based ultrasound-assisted extraction of marrubium vulgare linnand comparative evaluation of extracts for furan labdane diterpene(Marrubiin) concentration and antihypertensive potential. Current Bioactive Compounds. 2020; 16 (6): 924–936.

60. Wu XG, et al. Optimization of the preparation of nalmefene-loaded sustained-release microspheres using central composite design. Chemical and Pharmaceutical Bulletin. 2006; 54 (7): 977–981.
61. Udapurkar PP, et al.Diosmin Phytosomes: Development, Optimization and Physicochemical characterization. Indian J of Pharmaceutical Education and research. 2018; 52 (4S): S29-S36.
62. Lu M, et al. Phyto-phospholipid complexes (phytosomes): A novel strategy to improve the bioavailability of active constituents. Asian J Pharm Sci. 2019;14(3):265-274. doi: 10.1016/j.ajps.2018.05.011.
63. Nandhini S, et al. Development and characterization of a nano-drug delivery system containing vasaka phospholipid complex to improve bioavailability using quality by design approach. Res Pharm Sci. 2020;16(1):103-117. doi: 10.4103/1735-5362.305193.
64. Chaurasia, AK, et al. Synthesis, optimization, characterization, anti-oxidant and anti-cancerous activity analysis of nanoparticles IA-AuNPs synthesized using leaf extract of soil grown Ipomoea aquatica. Journal of Molecular Structure. 2025; *1327*: 141212.
65. Barani M, et al. Phytosomes as Innovative Delivery Systems for Phytochemicals: A Comprehensive Review of Literature. Int J Nanomedicine. 2021;16:6983-7022. doi: 10.2147/IJN.S318416.
66. Mirza MA, et al. Quercetin as a Therapeutic Product: Evaluation of Its Pharmacological Action and Clinical Applications-A Review. Pharmaceuticals (Basel). 2023;16(11):1631. doi: 10.3390/ph16111631.
67. Singh A, et al. Development and optimization of Andrographis paniculata extract-loaded phytosomes using Box-Behnken design approach. Journal of Integrated Science and Technology.2023; 11(4): 558.

Graphical Abstract: