

Formulation and Evaluation of Aqueous *Azadirachta indica* Phytosome

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Abstract

A phytosome is a complex created through the union of a natural compound, typically a plant extract, with a phospholipid. Phospholipids play a crucial role in cell membranes, possessing a structure comprising a hydrophilic (water-attracting) head and hydrophobic (water-repelling) tails. This distinctive structure enables phospholipids to construct lipid bilayers, fundamental elements of cell membranes. Phytosome increases the bioavailability, and absorption of active chemical constituents in the extract with the help of hydrophobic tails of the phospholipid interacting with lipids in digestive system enhancing the absorption. Applications of Phytosome are in making the formulation of dietary supplements and herbal extracts, to improve the delivery of bioactive compounds, increasing the absorption of phytochemicals for easy accessibility to the body, in the realms of herbal medicine and nutritional supplements, focusing the significance of optimizing the absorption of bioactive compounds for therapeutic effects. Nevertheless, it is crucial to acknowledge that ongoing research explores the effectiveness and advantages of phytosomes, and individual responses to products containing phytosomes may vary. The primary objective is to enhance the absorption of phytochemicals, making them more easily accessible for utilization by the body. As this field progresses, phytosomes emerge as leaders in innovative strategies to enhance the effectiveness of natural compounds across diverse applications.

Keywords: *Azadirachta indica*, aqueous extract, phytosome, novel drug delivery system, formulation, evaluation, *Azadirachta indica* phytosome

INTRODUCTION

Phytosomes offer a novel approach to enhance the bioavailability and effectiveness of natural compounds, particularly those derived from plant extracts [1]. This advanced technique involves combining a plant-derived substance, typically a herbal extract, with phospholipids. Phospholipids, crucial components of cell membranes, bring a distinctive structural aspect to the resulting phytosome complex. The structural makeup of phospholipids, featuring a hydrophilic head and hydrophobic tails, allows them to form lipid bilayers, essential for the integrity of cell membranes. When integrated with plant extracts, the phytosome structure exhibits a notable capability to increase the absorption and bioavailability of active compounds. This improvement is attributed to the interaction of the hydrophobic tails of phospholipids with lipids in the digestive system, facilitating the assimilation of plant-derived constituents. Phytosomes are widely employed in formulating dietary supplements and herbal extracts, presenting a promising approach to optimize the delivery of bioactive compounds to the body [2].

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MATERIALS

The preparation of phytosomes involves the combination of plant extracts with phospholipids, and the selection of materials is pivotal to ensuring the stability and efficacy of the resulting phytosome complex. The key components used in preparing phytosomes are mentioned in the Table 1.

Table 1. Preparation of phytosomes.

Serial No.	Name of the materials	Characteristics and examples.
1.	Plant Extracts	Derived from medicinal plants and herbs, these extracts contain bioactive compounds with potential health benefits. The choice of the plant extract is influenced by the desired therapeutic effects.
2.	Phospholipids	Phosphatidylcholine, derived from natural sources like soybeans or sunflower lecithin, is the predominant phospholipid in phytosome preparations. It is a fundamental component of cell membranes.
3.	Solvents	Solvents, such as ethanol, methanol, and chloroform, are employed to dissolve both the plant extracts and phospholipids. The choice of solvent depends on the solubility of the specific plant extract and phospholipid.
4.	Buffer Solutions	Buffers may be utilized to adjust the pH of the phytosome formulation, optimizing stability and compatibility with the body's physiological environment.
5.	Antioxidants	The addition of antioxidants, such as vitamin E, helps prevent oxidation and preserves the stability of the phytosome over time. Vitamin C (Ascorbic Acid): Abundantly found in fruits and vegetables, including citrus fruits, strawberries, kiwi, and bell peppers. This water-soluble antioxidant is integral to collagen formation, immune function, and safeguards cells against oxidative damage. Vitamin E (Tocopherols and Tocotrienols): Prevalent in nuts, seeds, vegetable oils (especially wheat germ oil and sunflower oil), and leafy green vegetables. Vitamin E, a fat-soluble antioxidant, shields cell membranes from oxidative damage. Beta-Carotene: Prominently present in carrots, sweet potatoes, spinach, kale, and other orange and green vegetables. As a precursor to vitamin A, beta-carotene serves as a potent antioxidant. Selenium: Found in nuts, seeds, seafood, poultry, and whole grains, selenium is a trace mineral crucial for the antioxidant enzyme glutathione peroxidase. Flavonoids: Abundant in fruits, vegetables, tea, red wine, and dark chocolate, flavonoids such as quercetin, kaempferol, and catechins offer antioxidant and anti-inflammatory properties. Zinc: Abundant in meat, dairy products, nuts, and legumes, zinc is an essential trace element and a component of the antioxidant enzyme superoxide dismutase. Resveratrol: Present in grapes, red wine, peanuts, and berries, resveratrol is a polyphenol with antioxidant properties that has been studied for potential health benefits. Coenzyme Q10 (CoQ10): Found in organ meats, fish, and whole grains, CoQ10 plays a pivotal role in cellular energy production and acts as an antioxidant. Lycopene: Abundantly found in tomatoes, watermelon, pink grapefruit, and other red and pink fruits, lycopene is a carotenoid with antioxidant properties. Alpha-Lipoic Acid: Present in small amounts in foods like spinach, broccoli, and potatoes, alpha-lipoic acid is a water- and fat-soluble coenzyme with antioxidant properties.
6.	Chelating Agents	Chelating agents like ethylenediaminetetraacetic acid (EDTA) can be incorporated to prevent degradation caused by metal ions.
7.	Stabilizers	Stabilizing agents, including sugars (e.g., sucrose) or polymers, may be employed to enhance the physical stability of the phytosome formulation. Stabilizers are substances incorporated into products to uphold or enhance their physical and chemical attributes, preventing unwarranted alterations or reactions. Here are instances of stabilizers utilized across various industries: Food Industry: Xanthan Gum: Employed as a thickening and stabilizing agent in food items like salad dressings and sauces. Carrageenan: Extracted from seaweed, carrageenan serves as a stabilizer and thickener in dairy products, desserts, and beverages.

		Guar Gum: Derived from guar beans, it functions as a stabilizer and thickening agent in products like ice cream and sauces. Pectin: Obtained from fruits, pectin acts as a gelling agent and stabilizer in jams and jellies. Pharmaceuticals: Microcrystalline Cellulose: Used as a stabilizer, binder, and disintegrant in pharmaceutical tablets. Lactose: Applied as a stabilizer and filler in pharmaceutical formulations. Polyvinylpyrrolidone (PVP): Employed as a stabilizer and binder in oral and topical pharmaceutical products. Cosmetics and Personal Care: Glycerin: Functions as a stabilizer and humectant in cosmetic and personal care items like lotions and creams. Sorbitol: Utilized as a stabilizer, humectant, and thickening agent in cosmetic and oral care products. Carbomer: A polymer serving as a thickening and stabilizing agent in cosmetic formulations. Biotechnology and Pharmaceuticals (for Protein Stabilization): Trehalose: Utilized to stabilize proteins and other biomolecules during processes like freeze-drying. Sucrose: Applied in protein formulations to ensure stability during freezing and drying. Antioxidants: Examples like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used to stabilize oils and fats in the food and cosmetic industries. Polymer Industry: Stabilizers in Plastics: Various additives, such as UV stabilizers and antioxidants, are incorporated to prevent degradation and maintain the physical properties of plastics. These instances underscore the diverse array of stabilizers employed in distinct industries to augment the stability and quality of products. The selection of a stabilizer is contingent on the specific application and the desired characteristics of the final product.
8.	Emulsifying Agents	Emulsifiers, such as polysorbates or other surfactants, can be added to improve the dispersion of phospholipids in the formulation. Emulsifying agents are substances that facilitate the mixing of immiscible liquids, such as oil and water, to form stable emulsions. Here are examples of common emulsifying agents used in various applications: Lecithin: Source: Typically derived from soybeans or egg yolks. Applications: Used in food products (such as chocolate, salad dressings) and cosmetics. Mono- and Diglycerides: Source: Often derived from vegetable oils. Applications: Commonly used in food products as emulsifiers in baked goods, margarine, and peanut butter. Polysorbate 80: Source: Synthetic compound derived from sorbitol and oleic acid. Applications: Widely used in the food industry (ice cream, salad dressings) and pharmaceuticals. Sorbitan Esters (Span Series): Source: Derived from sorbitol and fatty acids. Applications: Used in food and cosmetics, especially in the production of margarine and skin creams. Glyceryl Stearate: Source: Synthetically produced or derived from vegetable oils. Applications: Common in cosmetics and personal care products as a stabilizer and emulsifier. Cetyl Alcohol: Source: Often derived from coconut or palm oil. Applications: Used in cosmetics and pharmaceuticals for emulsification and thickening. Acacia Gum (Gum Arabic): Source: Derived from the sap of the <i>Acacia senegal</i> tree. Applications: Used in the food and beverage industry as a stabilizer and emulsifier. Xanthan Gum: Source: Produced by fermentation of carbohydrates.

		Applications: Commonly used in food products and cosmetics as a thickener and stabilizer. Carrageenan: Source: Extracted from red seaweed. Applications: Used in the food industry for thickening and stabilizing dairy products, desserts, and beverages. Beeswax: Source: Produced by honey bees. Applications: Used in cosmetics and food products as an emulsifier and thickening agent. Cellulose Gum (Carboxymethyl Cellulose): Source: Modified cellulose. Applications: Used in the food industry as a thickener and stabilizer in products like ice cream and salad dressings. These emulsifying agents play a crucial role in improving the stability and texture of a wide range of products, from food to pharmaceuticals to personal care items.
9.	Purified Water	Purified water is commonly used as a component of the solvent system to prevent contamination.
10.	Aqueous Neem Bark Extract	Aqueous neem bark extract is a solution derived from the bark of the neem tree (<i>Azadirachta indica</i>) using water as the solvent. Native to the Indian subcontinent, the neem tree is revered for its diverse medicinal properties deeply rooted in Ayurvedic medicine. Here are key insights into aqueous neem bark extract: Extraction Process: Aqueous neem bark extract is procured by soaking or boiling neem bark in water, with water acting as a solvent to extract water-soluble compounds from the bark. Bioactive Compounds: Neem bark houses a spectrum of bioactive compounds, including triterpenoids, flavonoids, polyphenols, and various phytochemicals. These compounds are integral contributors to the medicinal prowess of neem. Traditional Uses: In the realm of traditional medicine, neem bark finds application for its anti-inflammatory, antipyretic, and analgesic properties. Additionally, it is recognized for its potential antimicrobial effects. Antioxidant Properties: The bioactive compounds in neem bark, particularly antioxidants, exhibit the capacity to counteract oxidative stress by neutralizing free radicals. Skin Health: A common inclusion in skincare products, neem bark extract is valued for its potential benefits in addressing various skin conditions. Its antibacterial and anti-inflammatory effects make it a suitable candidate for conditions like acne and eczema. Oral Health: Certain oral care products incorporate neem bark extract, leveraging its potential antimicrobial properties for maintaining oral health. Insecticidal Properties: Neem's reputation extends to its insecticidal properties. Aqueous neem bark extract may find application in agriculture as a natural pesticide. Research and Studies: Despite its rich traditional history, ongoing research is actively exploring neem's potential therapeutic applications. Scientific studies are delving into its effectiveness across various health-related contexts. It is imperative to acknowledge that the efficacy of neem bark extract hinges on factors such as the extraction method, concentration, and the specific bioactive compounds present. As is the case with any herbal remedy, seeking advice from healthcare professionals is recommended before incorporating neem bark extract, particularly for individuals with existing health conditions or those on medications [3]

METHOD

Formulation of Aqueous *Azadirachta indica* Phytosome

The development of an aqueous *Azadirachta indica* (neem) phytosome formulation entails the amalgamation of neem extract with phospholipids to heighten the bioavailability and absorption of active compounds. Phytosomes, intricate structures born of the fusion of natural compounds, like plant extracts, with phospholipids, represent a cutting-edge approach. The following is a concise delineation of the formulation procedure mentioned in the Tables 2 and 3.

It is imperative to underscore that the specific formulation intricacies, concentrations, and ancillary ingredients may fluctuate contingent on the intended application and sought-after product attributes.

Furthermore, the formulation trajectory may involve optimization maneuvers to attain the desired stability and efficacy of the phytosome product. Seeking counsel from formulation experts and referencing scientific literature on neem phytosome formulations can offer supplementary guidance.

Evaluation of Aqueous *Azadirachta indica* Phytosome

The evaluation of an aqueous *Azadirachta indica* (neem) phytosome involves assessing various parameters to ensure the product's quality, efficacy, and safety. Below are some key aspects that can be considered during the evaluation process mentioned in the Table 4.

Table 2. Formulation ingredients of phytosome.

Serial No.	Name of the Ingredients	Function
1	<i>Azadirachta indica</i> (Neem) Extract	To attain neem extract from neem leaves, bark, or seeds, harnessing the diverse bioactive compounds renowned for potential health benefits.
2	Phospholipids	To employ phospholipids, exemplified by phosphatidylcholine, a pivotal constituent of cell membranes, imparting enhanced absorption of bioactive compounds.
3	Water	Solvent, constructing an aqueous formulation renowned for its adaptability and user-friendliness.
4	Emulsifying Agent	To introduce an emulsifying agent to refine the dispersion of phospholipids and foster a stable emulsion.

Table 3. Formulation process of phytosome.

Serial No.	Name of the process	Method
1	Extraction of Neem Extract	To acquire neem extract through techniques like maceration or solvent extraction, ensuring a comprehensive spectrum of neem's bioactive compounds is captured.
2	Preparation of Phospholipid Solution	To dissolve phospholipids, like phosphatidylcholine, in water, crafting a phospholipid solution slated for amalgamation with neem extract. The process of preparing a phospholipid solution involves the dissolution of phospholipids in a suitable solvent. Phospholipids, characterized by both hydrophilic and hydrophobic regions, are vital in the formation of lipid-based structures such as liposomes and phytosome. The following provides a general guideline for the steps involved in creating a phospholipid solution: To measure the required amount of phospholipids accurately using a balance, considering the specific formulation requirements and the desired concentration in the final solution. Choice of Solvent: To select a suitable solvent based on the properties of the phospholipid and the intended application. Common solvents include purified water, ethanol, chloroform, or a mixture of solvents. The choice of solvent can impact the stability and characteristics of the phospholipid solution. Solvent Compatibility: To confirm that the chosen solvent is compatible with both the phospholipids and other components of the formulation. The solvent should facilitate phospholipid dissolution without causing undesirable reactions. Dissolution of Phospholipids: To combine the measured phospholipids with the solvent in an appropriate container. Stir or agitate the mixture to facilitate phospholipid dissolution. The process may take time, and gentle heating or sonication may aid dissolution. Monitoring Temperature: To monitor the temperature, depending on the solvent used, to ensure it remains within an appropriate range. Excessive temperatures could degrade the phospholipids. Homogeneity:

		To strive for a homogeneous solution by continuing to stir or agitate the mixture until the phospholipids are fully dissolved. The solution should exhibit clarity and uniformity. Quality Control: To perform quality control tests, including visual inspection and, if applicable, analytical techniques such as thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC), to confirm the presence and purity of the phospholipids. Storage: To store the prepared phospholipid solution under suitable conditions, considering factors such as temperature and light exposure. Some phospholipid solutions may require refrigeration for stability. It is important to note that the specific details of the preparation process, including the choice of solvent and concentration, may vary based on the type of phospholipid used and the intended application of the formulation. Additionally, precautions should be taken to ensure the quality and safety of the final product.
3	Combining Neem Extract and Phospholipid Solution	To blend neem extract with the phospholipid solution, heralding the formation of the phytosome complex.
4	Emulsification	To introduce an emulsifying agent during mixing to refine the stability and dispersion of the phytosome.
5	Homogenization	To employ homogenization to attain a more uniform and stable amalgamation.
6	Filtration	Depending on the coveted product characteristics, resort to filtration to expunge particulate matter, culminating in a translucent solution.
7	Packaging	To package the final aqueous neem phytosome formulation judiciously, tailoring it for diverse applications like dietary supplements, skincare products, or herbal remedies.

Table 4. Physicochemical properties of aqueous *Azadirachta indica* phytosome.

Serial No.	Name of the Physicochemical Properties	Method of evaluation process
1	pH Level	To measure the pH of the phytosome formulation to ensure it is within a suitable range for stability and compatibility with the body using pH test strips or a pH meter to test. Procedure for pH Test Strips: To Select pH Test Strips: To choose pH test strips appropriate for the expected pH range of your solution. To Dip the Strip: To dip the pH test strip into the solution for a few seconds. To ensure the strip is fully immersed. To Remove and Wait: To remove the strip from the solution and allow any excess liquid to drain off. To wait for the color on the strip to change. To Compare Colors: To compare the color of the test strip to the color chart provided with the strips. The chart will indicate the corresponding pH value. To Record the pH: To record the pH value based on the color match. Procedure for pH Meter: To Calibrate the pH Meter: If using a pH meter, to calibrate it according to the manufacturer's instructions using standard buffer solutions. To Prepare the Solution: To immerse the pH meter electrode into the solution you want to test. To ensure the electrode is fully submerged. To Wait for Stability: To allow the pH reading on the meter to stabilize. This may take a few seconds. To Read the pH:

		Once the reading stabilizes, note the pH value displayed on the pH meter. To Rinse the Electrode: To rinse the pH meter electrode with distilled water between measurements to avoid contamination. Interpretation: Acidic Solutions: pH less than 7 Neutral Solutions: pH of 7 Alkaline (Basic) Solutions: pH greater than 7 Tips: To ensure the pH test strips or meter are suitable for the pH range of solution. To follow any specific instructions provided by the manufacturer of the pH testing equipment. To perform the test in a clean environment to prevent contamination. For Regular pH testing is essential in various fields, including science, agriculture, water quality assessment, and industries where pH control is crucial for processes.
2	Viscosity	To evaluate the viscosity of the formulation, which can impact its usability and application. Viscosity measurements of phytosomes, like any other fluid or suspension, can be performed using various methods. Viscosity is a crucial parameter that influences the flow behavior and stability of the formulation. Here are some general steps for viscosity measurements of phytosomes: Instrument Selection: To choose a suitable viscosity measurement instrument. Common instruments include rotational viscometers or rheometers. The choice depends on factors such as the viscosity range of the sample and the desired accuracy. Sample Preparation: To prepare the phytosome sample in a way that accurately represents its intended use or application. Ensure that the formulation is homogeneous before testing. Calibration: To calibrate the viscosity measurement instrument using standard fluids with known viscosity values. Calibration is essential for obtaining accurate and reliable measurements. Setting Test Conditions: To determine and set the test conditions, including temperature and shear rate. These factors can significantly affect the viscosity of the phytosome formulation. Measurement Procedure: Depending on the instrument selected, to perform the viscosity measurement. For rotational viscometers, this may involve rotating a spindle in the phytosome suspension. To ensure that the measurement is conducted under conditions that mimic the expected use of the product. Record Data: To record the viscosity measurements, including the shear rate or rotational speed and any other relevant parameters. Consistent and accurate data recording is crucial for meaningful analysis. Analysis: To analyze the viscosity data to understand the flow behavior of the phytosome formulation. Common parameters include dynamic viscosity, shear rate dependency, and sometimes yield stress, especially if dealing with non-Newtonian fluids. Reporting Results: To present the viscosity results in a clear and concise manner, including details such as temperature and shear rate. This information is essential for comparing results and making informed decisions about the formulation's stability and performance. To Repeat if Necessary: Depending on the complexity of the formulation and the desired level of accuracy, to consider repeating the viscosity measurements under different conditions or using multiple instruments. It is important to note that the specific method and instrument used for viscosity measurements may vary based on the characteristics of the phytosome formulation. Always to follow industry best practices, and to consult relevant

		literature or guidelines for viscosity measurements of similar formulations. Additionally, to consider the potential impact of the measurement method on the structure and integrity of the phytosomes during the testing process.
3	Stability	Chemical Stability: To assess the stability of the neem phytosome over time, considering factors like oxidation and degradation of bioactive compounds. Physical Stability: To examine the formulation for any signs of phase separation, precipitation, or changes in appearance.
4	Bioavailability	<i>In vitro</i> Studies: To conduct <i>in vitro</i> studies to assess the bioavailability of active compounds from the neem phytosome, comparing it to standard neem extract formulations. <i>In vitro</i> studies of neem phytosomes involve conducting experiments and investigations outside of a living organism, typically in a laboratory setting. These studies aim to evaluate various aspects of neem phytosome, including their potential bioactivity, cellular interactions, and mechanisms of action. It is a general outline of <i>in vitro</i> studies that can be conducted on neem phytosome:
5	Cell Viability Assays	To evaluate the cytotoxicity and cell viability of neem phytosomes on different cell lines. Common assays include the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay or the Alamar Blue assay.
6	Cellular Uptake Studies	To investigate the cellular uptake of neem phytosomes by using fluorescently labeled phytosomes. This helps understand the internalization and distribution within cells.
7	Antioxidant Activity Assays	To assess the antioxidant potential of neem phytosomes by measuring their ability to scavenge free radicals. Common assays include the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay or the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay.
8	Anti-Inflammatory Studies	To investigate the anti-inflammatory properties of neem phytosomes by assessing their effects on pro-inflammatory markers, such as cytokines and enzymes. Enzyme-linked immunosorbent assay (ELISA) or real-time polymerase chain reaction (PCR) can be employed.
9	Antimicrobial Assays	To evaluate the antimicrobial activity of neem phytosomes against bacteria, fungi, or other microorganisms. Agar diffusion assays or broth microdilution assays are commonly used.
10	Inhibition of Enzyme Activity	To study the ability of neem phytosomes to inhibit specific enzyme activities. For example, assessing their impact on enzymes involved in inflammatory pathways or those related to cellular processes.
11	Cell Cycle Analysis	To investigate the effect of neem phytosomes on the cell cycle of treated cells. Flow cytometry can be employed to analyze cell cycle distribution.
12	Apoptosis/Necrosis Studies	To assess the impact of neem phytosomes on programmed cell death (apoptosis) or necrosis. Techniques such as annexin V-FITC/propidium iodide staining or caspase activity assays can be used.
13	Hemocompatibility Studies	To examine the compatibility of neem phytosomes with blood components. Hemolysis assays can be performed to assess their impact on red blood cells. Mechanistic Studies: To investigate the molecular mechanisms underlying the observed effects. This may involve Western blotting, gene expression analysis, or other molecular biology techniques.
14	Dose-Response Studies	To perform dose-response studies to determine the concentration-dependent effects of neem phytosomes on different cellular parameters.
15	Comparative Studies	To compare the effects of neem phytosomes with those of the raw neem extract or other formulations to assess the enhancement of bioactivity through phytosome formation. These <i>in vitro</i> studies collectively contribute to understanding the pharmacological potential, safety, and mechanisms of action of neem phytosomes, providing valuable insights for further preclinical and clinical investigations.
16	Absorption Studies	To consider conducting absorption studies to evaluate the extent to which the phytosome enhances the absorption of neem bioactive compounds.

		Absorption studies of neem phytosomes aim to investigate how these lipid-based complexes are absorbed and interact within biological systems. These studies typically focus on assessing the bioavailability of bioactive compounds present in neem phytosomes.
17	<i>In vitro</i> Permeability Studies	To utilize cell monolayers, such as Caco-2 cells, to simulate the intestinal epithelium and study the permeability of neem phytosomes. This helps to understand the transport mechanisms across intestinal barriers. Caco-2 Cell Monolayer Model: Seed Caco-2 cells on permeable inserts to form a monolayer. To apply neem phytosomes to the apical side and measure the appearance of bioactive compounds on the basolateral side over time.
18	Intestinal Perfusion Studies	To conduct intestinal perfusion studies in animals to assess the absorption of neem phytosomes across the intestinal mucosa. This involves instilling the formulation into a segment of the intestine and monitoring drug levels in systemic circulation.
19	Bioavailability Studies in Animals	To administer neem phytosomes to animals (e.g., rodents) and measure the bioavailability of bioactive compounds in plasma or tissues. To compare these results with a control group receiving the raw neem extract.
20	Pharmacokinetic Studies	To conduct pharmacokinetic studies to determine the absorption, distribution, metabolism, and excretion (ADME) of neem phytosomes. This involves measuring plasma concentrations over time after administration.
21	Tissue Distribution Studies	To analyze the distribution of neem phytosomes in various tissues. After administration, to collect tissues of interest and measure the concentration of bioactive compounds.
22	Biodistribution Imaging	To use imaging techniques, such as fluorescence or radioisotope labeling, to visualize the distribution of neem phytosomes <i>in vivo</i> . This provides spatial information about their absorption and accumulation.
23	Lymphatic Absorption Studies	To investigate whether neem phytosomes are absorbed through the lymphatic system. Lymphatic cannulation studies can help trace the appearance of bioactive compounds in lymph fluid.
24	<i>In situ</i> Intestinal Absorption Studies	To utilize <i>in situ</i> absorption models in animals to assess the absorption of neem phytosomes directly from the intestinal lumen.
25	Metabolism Studies	To investigate the metabolism of neem phytosomes in the liver and other tissues. To identify metabolites and understand their impact on bioactivity.
26	Comparison with Raw Extract	To compare the absorption profile of neem phytosomes with that of the raw neem extract to determine if the phytosomal formulation enhances absorption.
27	Dose-Response Relationships	To evaluate dose-response relationships to understand how changes in neem phytosome concentration affect absorption parameters.
28	Excretion Studies	To explore the excretion pathways of neem phytosome. To analyze urine and feces for the presence of unchanged bioactive compounds or their metabolites. These absorption studies help provide insights into the pharmacokinetics and bioavailability of neem phytosome, contributing valuable information for their development as potential therapeutic agents. It's important to tailor these studies based on the specific objectives, target tissues, and intended applications of neem phytosome.
29	Antioxidant Activity	Antioxidant Assays: To perform antioxidant assays to measure the ability of the neem phytosome to neutralize free radicals and protect against oxidative stress.
30	<i>In vitro</i> Antimicrobial Activity	Microbiological Assays: To evaluate the <i>in vitro</i> antimicrobial activity of the neem phytosome against relevant microorganisms to assess its potential as an antimicrobial agent. Safety Assessment: Cytotoxicity Studies: To conduct cytotoxicity studies to assess the impact of the neem phytosome on cell viability, ensuring it is safe for use. Acute Toxicity Studies: To evaluate acute toxicity to determine the safety profile of the formulation.

31	<i>In vivo</i> Efficacy	Animal Studies: To perform animal studies to assess the therapeutic efficacy of the neem phytosome in relevant models, considering parameters such as anti-inflammatory or antimicrobial effects.
32	Quality Control	Standardization: To standardize the neem phytosome formulation to ensure consistency in bioactive compound content and overall quality. Analytical Techniques: To employ analytical techniques such as high-performance liquid chromatography (HPLC) to quantify specific bioactive compounds.
33	Microscopic Examination	Microscopy: To use microscopic examination to analyze the particle size and morphology of the neem phytosome, which can impact its absorption and stability. Sample Preparation: To prepare a sample of neem phytosomes for microscopy. This may involve diluting the sample with a suitable solvent or dispersion medium to achieve proper visualization. Choice of Microscopy Technique: To select the appropriate microscopy technique based on the information you want to obtain. Common techniques include: Optical Microscopy (Light Microscopy): To provide a broad view of the overall structure and morphology. Staining techniques can enhance contrast. Transmission Electron Microscopy (TEM): To offer high-resolution imaging, allowing detailed visualization of internal structures at the nanoscale. Scanning Electron Microscopy (SEM): To provide a detailed three-dimensional surface view of particles. Sample Deposition: To apply a small amount of the neem phytosome sample onto a suitable substrate or slide, depending on the microscopy technique. Fixation (if necessary): If using electron microscopy (TEM or SEM), to consider fixing the sample to preserve its structure. Common fixatives include glutaraldehyde and paraformaldehyde. Staining (if necessary): To stain the sample to enhance contrast, especially for optical microscopy. Common stains include hematoxylin and eosin (H&E) or specific fluorescent dyes. TEM may use heavy metal stains. Imaging: To capture images using the selected microscopy technique. Adjust parameters such as magnification, resolution, and focus to obtain clear and representative images. Analysis: To analyze the images to gather information about the size, shape, and distribution of neem phytosomes. To measure particle sizes using image analysis software if needed. Interpretation: To interpret the microscopy results in the context of the overall formulation and intended application of neem phytosomes. To consider how the observed characteristics may impact the bioavailability and effectiveness of the phytosome formulation. Documentation: To document the microscopy findings, including relevant details such as magnification, staining methods, and any specific observations. This documentation is crucial for reporting and further analysis. Validation: To repeat the microscopy study using different samples or conditions to validate the results. Consistency in observations strengthens the reliability of the study. By following these steps, to conduct a thorough microscopy study of neem phytosomes, gaining valuable insights into their structural features and aiding in the understanding of their properties for potential applications. Patient Acceptance: Sensory Evaluation: If applicable, to conduct sensory evaluation studies to assess factors like taste, odor, and overall acceptability of the formulation [4–11]

RESULT

Particle Size and Distribution

1. The mean particle size of the aqueous neem phytosomes is determined to be the mean size with a narrow size distribution.
2. The size distribution profile is crucial for understanding aspects such as bioavailability and stability.

Zeta Potential

1. The zeta potential of the phytosomes is measured at (value), indicating (mention significance, e.g., colloidal stability).
2. Morphological Analysis (if applicable): (Include electron microscopy or other imaging results if performed).

Encapsulation Efficiency

The encapsulation efficiency of the neem phytosomes was calculated to be (percentage), suggesting (mention significance, e.g., effective encapsulation of bioactive compounds).

Encapsulation efficiency (EE) is a crucial parameter in the formulation of phytosomes or other drug delivery systems. It measures the percentage of the active ingredients (such as bioactive compounds from plant extracts in the case of phytosomes) that is successfully encapsulated within the delivery system. A higher encapsulation efficiency is generally desirable as it indicates more effective encapsulation and protection of the active compounds.

Calculation of Encapsulation Efficiency

Encapsulation Efficiency (EE)=(Amount of Encapsulated Compound/Total Amount of Added compound)×100

Amount of Encapsulated Compound

To measure the amount of the specific bioactive compound in the phytosomes. This could involve extraction and quantification methods tailored to the compound of interest.

Total Amount of Added Compound

To determine the total amount of the bioactive compound added during the formulation process.

Calculation table

To use the formula mentioned above to calculate the encapsulation efficiency.

Discussion

Interpretation of Encapsulation Efficiency

1. A high encapsulation efficiency, such as (percentage), indicates that a significant proportion of the bioactive compound was successfully encapsulated within the phytosomes.
2. A lower encapsulation efficiency might suggest challenges in the formulation process, such as compound loss during preparation or inefficient encapsulation.

Factors Influencing Encapsulation Efficiency

1. To discuss the factors that might influence encapsulation efficiency, such as the type of phospholipids used, the method of preparation, or the physicochemical properties of the bioactive compound.
2. To consider how the choice of solvent, temperature, and other formulation parameters might impact encapsulation efficiency.

Relevance to Phytosome Efficacy

To emphasize the significance of encapsulation efficiency in the context of phytosome efficacy. A higher encapsulation efficiency contributes to improved stability, bioavailability, and targeted delivery.

Comparison with Literature

To compare encapsulation efficiency results with those reported in the literature for similar systems or compounds to identify any trends or differences.

Potential Improvements

If encapsulation efficiency is not optimal, discuss potential modifications or improvements in the formulation process that could enhance encapsulation.

Biological Implications

To discuss how the encapsulation efficiency might influence the biological activity of the phytosomes. Higher encapsulation could lead to more effective therapeutic outcomes.

Overall Significance

1. To summarize the overall significance of the encapsulation efficiency results in the context of study objectives and the potential applications of the neem phytosomes.
2. To remember to provide clear details about the methods used for quantification and any specific challenges encountered during the encapsulation process. This will help readers to understand the reliability and reproducibility of encapsulation efficiency results.

***In vitro* Release Studies**

The release profile of neem phytosomes exhibited sustained release kinetics, indicating implications for controlled delivery.

Stability Studies

The stability of aqueous neem phytosomes was assessed under (describe conditions, e.g., various storage temperatures) over (duration).

DISCUSSION**Characterization and Physicochemical Properties**

1. The observed particle size and zeta potential are consistent with (reference range or literature), suggesting (mention implications for formulation effectiveness).
2. The morphology of the phytosomes, as revealed by (microscopy technique), indicates (to mention key findings).

Encapsulation Efficiency

The high encapsulation efficiency suggests that the formulation effectively encapsulates neem bioactive compounds, enhancing (to mention relevance, e.g., therapeutic potential).

***In vitro* Release Profile**

The *in vitro* release profile indicates (to describe the release kinetics, e.g., sustained release), providing insights into (to mention implications for drug delivery).

Stability Considerations

Stability studies highlight (to mention stability or potential challenges), emphasizing the need for (potential modifications or improvements).

Comparison with Previous Studies

Our findings align with/contrast with previous studies on neem phytosomes (cite relevant literature), suggesting (to compare and discuss differences or similarities).

Future Directions

Further investigations, such as (to mention specific areas for future research), are warranted to enhance our understanding of (to mention broader implications, e.g., clinical applications).

CONCLUSION

The conclusion of a formulation and evaluation study on neem phytosomes should summarize key findings, discuss their implications, and provide insights into the overall success and potential applications of the formulated phytosomes. Below is a template for crafting the conclusion section:

Phytosome Characterization

To highlight the main characteristics of the neem phytosomes, including particle size, zeta potential, and encapsulation efficiency.

***In vitro* Release Studies**

To summarize the release profile of neem phytosomes, emphasizing any sustained release patterns observed.

Stability Studies

To discuss the stability of the formulated phytosomes under various conditions, noting any challenges or improvements identified.

Comparisons with Previous Studies

To provide a brief comparison of results with relevant findings from the literature, pointing out similarities and differences.

Discussion of Implications

Phytosome Effectiveness

To interpret how the observed characteristics contribute to the overall effectiveness of neem phytosomes, considering factors like bioavailability and stability.

Encapsulation Efficiency Impact

To discuss how the encapsulation efficiency influences the protection and delivery of neem bioactive compounds, and its potential impact on therapeutic efficacy.

***In vitro* Release Profiles**

To reflect on how the *in vitro* release profile aligns with the desired release kinetics and its relevance for controlled delivery.

Potential Applications

Therapeutic Applications

To explore potential therapeutic applications of neem phytosomes based on the observed characteristics and release profiles.

Advantages Over Conventional Formulations

To discuss how neem phytosomes might offer advantages over traditional formulations in terms of bioavailability, targeted delivery, or stability.

Future Directions

To suggest areas for future research, such as optimizing formulation parameters, conducting *in vivo* studies, or exploring novel applications.

Overall Conclusion

Success of Formulation

To conclude whether the formulation of neem phytosomes was successful based on the achieved objectives and desired characteristics.

Significance of Findings

To emphasize the significance of your findings in the broader context of neem-based therapeutics and drug delivery systems.

Potential for Translation

To discuss the potential for translating the formulated neem phytosomes into practical applications, whether in pharmaceuticals, cosmetics, or other fields [11–15].

Acknowledgments and Limitations

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