

DEVELOPMENT AND INVITRO CHARACTERIZATION OF PHYTOSOMES OF THE BOBGUNNIA MADAGASCARIENSIS WITH POOR BIOAVAILABILITY

Yash Nagar* and Dr. Chetan Singh Chauhan

Bhupal Nobles' Institute of Pharmaceutical Sciences, B N University, Udaipur (Raj.) 313001.

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*Corresponding Author

Yash Nagar

Bhupal Nobles' Institute of
Pharmaceutical Sciences, B
N University, Udaipur (Raj.)
313001.

ABSTRACT

The study focuses on the creation and laboratory analysis of phytosomes produced from the Bobgunniamadagascariensis plant, which has medicinal benefits but is limited by its low absorption. The major goal is to boost the absorption of active phytoconstituents by the use of advanced drug delivery methods, especially phytosomes. The study uses multiple ways to make and measure the phytosomes, including liquid evaporation, lyophilization, and mechanical dispersion methods. The resultant phytosomal complexes are submitted to strict analysis methods, such as Fourier Transform Infrared Spectroscopy (FTIR), Transmission Electron Microscopy (TEM), and in-vitro drug release tests. The results show a considerable rise in the solubility and absorption of the active ingredients when enclosed in phytosomes compared to their free forms. Phytosomes show better stability, greater

drug-trapping effectiveness, and longer drug-release patterns. These results indicate that the phytosomal preparation of Bobgunniamadagascariensis might serve as a valid method for beating the limits of traditional plant drug delivery systems, providing possible therapeutic benefits in the treatment of numerous diseases. In conclusion, the study gives proof that phytosomes may successfully boost the solubility of plant products, opening the door for future research and growth in this area. The successful application of phytosome technology in this study shows its promise as a feasible method for improving the delivery and effectiveness of plant-based treatments.

KEYWORDS: Phytosomes show better stability, greater drug-trapping effectiveness, and longer drug-release patterns.

INTRODUCTION

The use of plant drugs has a long past, especially in ancient systems of medicine such as Ayurveda, where they have been applied for thousands of years. Botanical medicines are drawn from plants and have formed the cornerstone of traditional healthcare systems throughout numerous cultures. The World Health Organization (WHO) believes that roughly 80-81% of the world population currently relies on plant drugs for healthcare. The rising global interest in plant drugs is driven by their perceived safety, effectiveness, and the reduced frequency of side effects compared to manufactured pharmaceuticals.

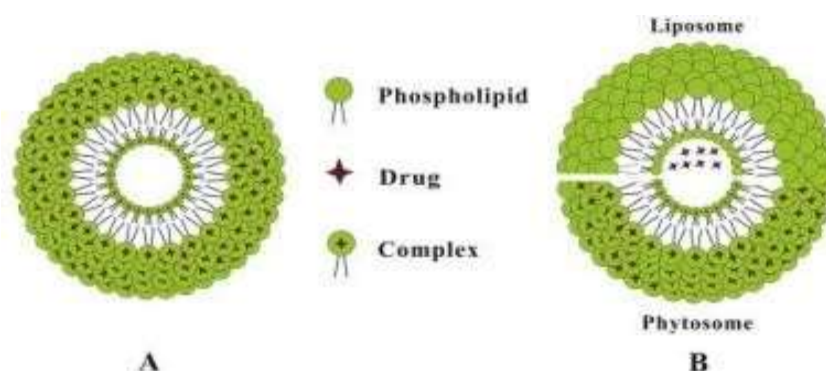


Figure 1.1: Schematic representation of the structure of phytosome (A) and difference between liposome and phytosome (B).

Botanical medicines are known for their natural origin, which adds to their biodegradability and prolonged release in the body. These qualities make them especially ideal for long-term treatment of chronic illnesses. Additionally, the use of plant treatments is cost-effective, making them available to a larger community. However, despite their benefits, plant medicines face significant hurdles, especially in terms of their absorption and the regularity of their active ingredients. The major limits of plant medicines include their big molecular size, poor fat solubility, and low absorption rates in the stomach system. These factors add to the lower absorption of the active chemicals, reducing their medicinal usefulness. Furthermore, plant medicines are often offered in bulk powder form, making them less handy for unit-dose delivery and less stable in the body's digestive environment. These problems show the need for improved drug delivery methods to increase the absorption and effectiveness of plant medicines.^[1]

In recent years, there has been a growing interest in creating new drug delivery methods to solve the limits of plant treatments. One such invention is the phytosome technology. Phytosomes are improved types of plant goods that improve the absorption of vegetable oils.

This method involves the complexation of plant products with phospholipids, making a lipid-compatible chemical complex that improves the intake of the active compounds in the body.^[2-3] Phytosomes offer several benefits over standard plant products, including better stability, focused release, and increased therapeutic effectiveness.



Figure 1.2: Diagrammatic representation of advantages of Phytosomes.

Phytosomes are made of a phospholipid molecule mixed with a regulated plant extract, producing a chemical that is better taken by the body. This unique transport method has been proven to greatly improve the bioavailability of several plant products, making them more useful in healing a variety of health problems. The better absorption and solubility given by phytosomes make them a possible method for solving the problems involved with traditional plant medicines.

The importance of this work lies in its ability to add to the creation of more effective plant medicine formulas. By improving the absorption and therapeutic usefulness of plant medicines, phytosome technology might play a vital part in growing the area of herbal medicine. This study wants to examine the production and in-vitro description of phytosomes made from *Bobgunniamadagascariensis*, a plant known for its medicinal benefits but limited by low absorption.^[4] The study aims to boost the absorption of active phytoconstituents via the application of phytosome technology, therefore offering a possible answer to the limits of

current plant drug delivery methods.

MATERIALS AND METHODS

This part starts by describing the pre-formulation studies, which involve the collecting and processing of the plant material, organoleptic research, production of standard solutions, and the building of a standard curve. The main material utilized in this study is the Bobgunniamadagascariensis extract, which was bought from Vital Herbs Pvt. Ltd., New Delhi, and further processed for analysis.

Materials

The study utilized many components, including phosphatidylcholine from Lipoid GmbH, Germany, methanol, isopropyl alcohol, potassium dihydrogen orthophosphate, and disodium hydrogen orthophosphate, among others [5m]. The plant material was gathered in powder form and put to several basic tests to check its features and suitability with other excipients utilized in the study.

List of the instruments

S. No.	Instruments	Manufacturer
1	UV/VIS Spectrophotometer,	Shimadzu, Japan
2	Weighing balance, (CY220)	Shimadzu, Japan
3	Particle Size Analyzer & Zeta Potential	Anton-Paar
4	Magnetic stirrer	Remi Equipments, Mumbai
5	Diffusion Cell Assembly	Orchid Scientific, Maharashtra
6	Vortex mixer	Remi (SLM-VM-3000), Bangalore
7	Hot air oven	PLT 125A/Tanco, Delhi
8	Viscometer	Anton-Paar
9	pH Meter	Ohaus, USA
10	Melting Point Apparatus	Remi Equipment, Mumbai
11	Infrared red spectrophotometer (FTIR)	Bruker Alpha, Berlin, Germany
12	Rotary Evaporator	Popular, India

List of the chemicals

S. No	Materials	Source
1	Bobgunnia madagascariensis	Vital Herbs Pvt. Ltd.
2	Phospholipid	Lipoid GmbH, Germany
3	Methanol	Fisher Scientific India Pvt. Ltd.
4	Iso propyl alcohol	Fisher Scientific India Pvt. Ltd.
5	Potassium Dihydrogen orthophosphate	Thomas Baker
6	Disodium hydrogen orthophosphate	Thomas Baker
7	HCl	SD Fine-chem. Ltd, Mumbai
8	n-octanol	SD Fine-chem. Ltd, Mumbai
9	Chloroform	Fisher Scientific India Pvt. Ltd.

10	DCM	Fisher Scientific India Pvt. Ltd.
11	Acetone	Fisher Scientific India Pvt. Ltd.

Pre-formulation Studies

Organoleptic Study: The organoleptic features of the *Bobgunniamadagascariensis* powder extract, such as color, taste, and texture, were studied by spreading a tiny sample on white paper for eye examination.

Preparation of Standard Solution: A standard solution of 1000 µg/ml was made by soaking about 100 mg of the pure extract in 100 ml of water in a volumetric flask. The solution was then sonicated for 10 minutes and reduced to the suitable amount for further research.

Preparation of Standard Curve: The standard curve was built by further reducing the stock solution to obtain several values ranging from 5-30 µg/ml. The absorbance of these solutions was recorded at 282 nm using a UV-visible spectrophotometer to plot the reference curve.^[5]

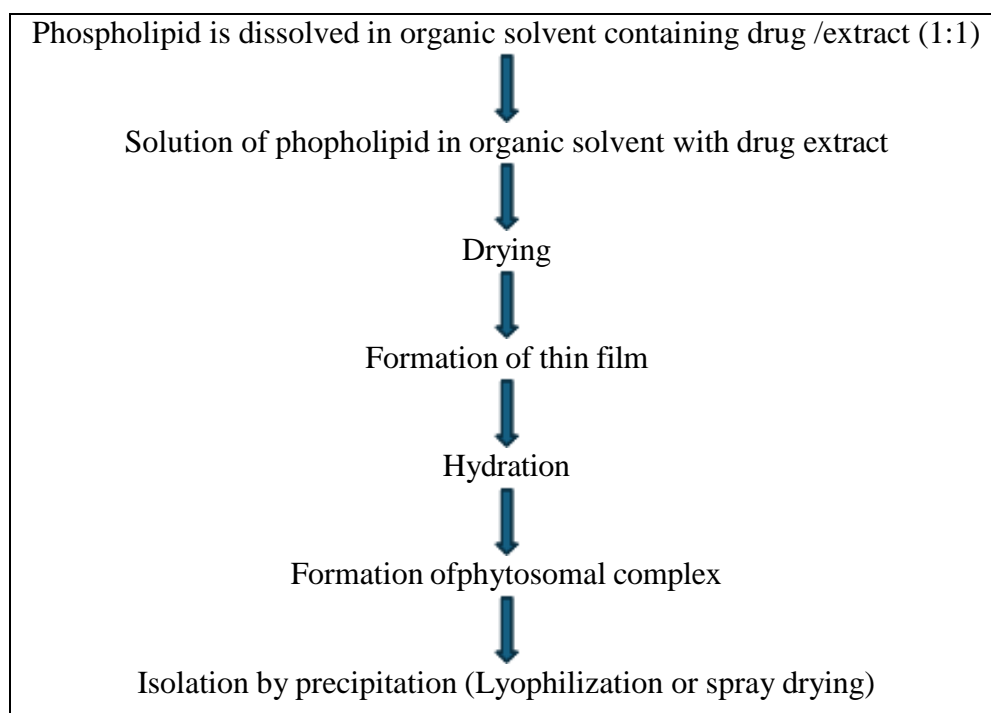


Figure 1.3: Common stages of phytosome preparation.

FTIR Analysis

The FTIR (Fourier Transform Infrared) spectroscopy was performed for structure study. It needed recording the infrared spectra of the extract and its mixing with excipients to find any interaction between the medicine and excipients. The study was done within the 4000 - 400 cm⁻¹ range, allowing the discovery of any chemical or physical interactions that could

change the formulation's stability.

Extraction and Analysis

The extraction of the *Bobgunniamadagascariensis* was carried out utilizing the Soxhlet extraction method. Approximately 200 g and 40 g of the powdered extract were treated to this process using specific liquids (methanol and water). After 4-5 rounds of extraction, the liquid and residue were left to dry at room temperature.^[6-8] The extracts were then kept at 4°C for further tests.

Phytochemical Screening

Preliminary phytochemical screening was performed to identify key chemicals such as alkaloids, flavonoids, glycosides, saponins, and steroids in the extract.

This was found by several qualitative tests:

Alkaloid Detection: Mayer's and Wagner's tests were performed to prove the presence of alkaloids.

Flavonoid Detection: Lead acetate and sulfuric acid tests were performed to identify flavonoids.

Steroid Detection: Steroids were discovered using a mixture of acetic anhydride and sulfuric acid, which produced a color shift from violet to blue or green.

Terpenoid Detection: The Salkowski test was used to identify terpenoids based on the look of a reddish-brown tint.

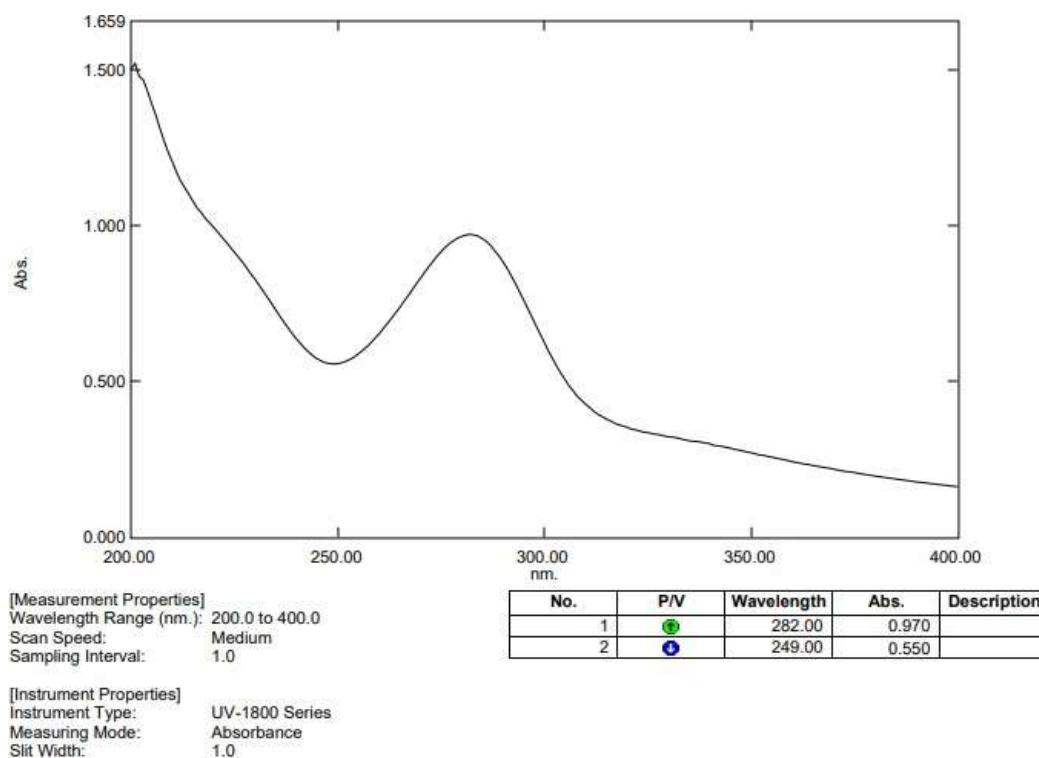
Anthraquinone Detection: The Borntrager's test was performed to prove the presence of anthraquinones by the production of a pink hue following a series of chemical reactions. This thorough method allowed the right discovery and description of the active components within the *Bobgunniamadagascariensis* extract, creating the basis for the study's future laboratory work and analysis.

RESULTS

The results of the study centered on the description and review of a BM extract-loaded complex phytosome product. The study started with preformulation studies, which comprised analyzing the physical and biological features of the extract. These studies showed that there

were no major obstacles to the creation of a successful dosage form. The organoleptic features of the extract were marked by a dark hue, bad smell, and harsh taste.^[9]

UV spectroscopy was performed to assess the absorption maximum of the *Bobgunniamadagascariensis* extract in water, which was found to be 282 nm. A calibration curve was made using different amounts of the extract, showing good uniformity with an R^2 value of 0.999, proving the stability of the spectroscopic method.



UV spectrum of *Bobgunniamadagascariensis* extract in water.

Name of drug	Absorption maxima (λ_{max})	
	Observed	Reference
<i>Bobgunnia madagascariensis</i>	282 nm	282 nm

The study also involved the production of a difficult phytosome mixture and its measurement using numerous measures. The visual look of the complex phytosome showed that a thin layer was made across all batches, and the percentage yield of the formulations ranged from $94.195 \pm 0.577\%$ to $96.165 \pm 0.367\%$, showing a fair yield across different formulations. The solubility tests were done in water and n-octanol, showing that the complex showed better solubility in DCM by 13% compared to the pure extract.^[10-11] The solubility in water ranged from 0.117 ± 0.001 to 1.238 ± 0.003 mg/mL, showing an improvement in solubility, which is an important factor for absorption. The solubility in n-octanol was also

improved, ranging from 0.047 ± 0.001 to 0.328 ± 0.003 mg/mL, which suggests a better spread in fatty media. The percentage drug capture of the BM extract-loaded complex phytosome was another important measure tried in the study. The entrapment efficiency ranged from $69.422 \pm 0.281\%$ to $94.621 \pm 0.136\%$, with the largest capture found in formulation F7, which showed a value of $94.621 \pm 0.136\%$. This result showed that raising the lipid content improved the drug trapping up to a certain limit.

Further study of the better version F7A4 included particle size distribution, zeta potential, and TEM inspection. The particle size of the F7A4 formulation was found to be 252.4 nm with a polydispersity index (PDI) of 0.234, showing a uniform particle size distribution. The zeta potential was found at -29.7 mV, showing the safety of the composition, which is important for the shelf life and efficiency of the drug delivery system.^[12] TEM scans showed that the BM extract-loaded complex phytosome was circular in form, further supporting the successful production of the necessary nanoparticles.

The FTIR study of the formulation showed that the spectra of the final formulation retained some of the usual peaks of the *Bobgunniamadagascariensis* extract, with small changes. This suggests that although the complexation changed the chemical environment, the important functional groups were preserved, ensuring the biological activity of the extract.^[13]

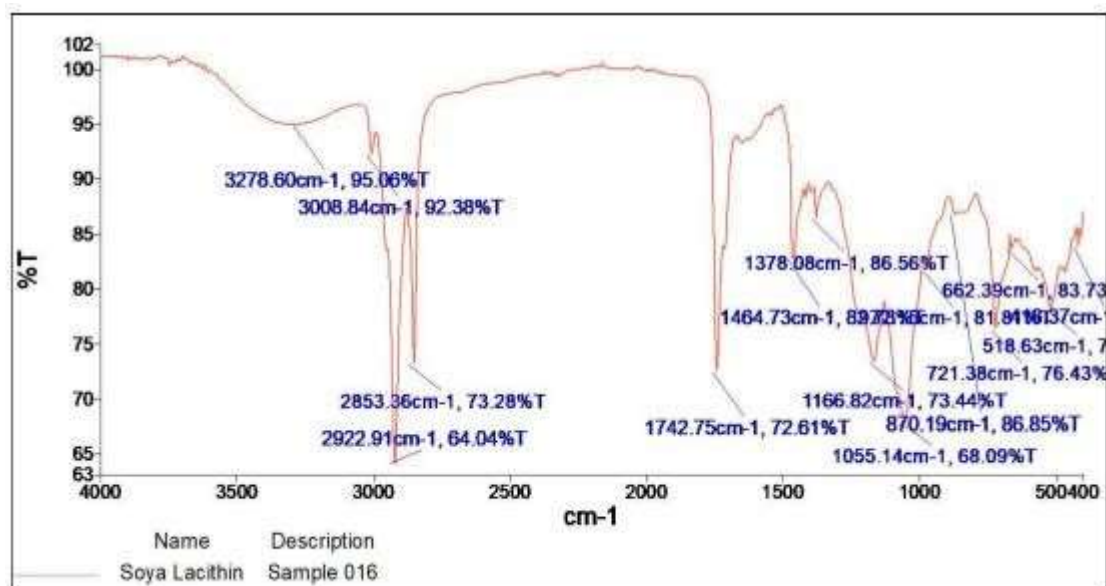
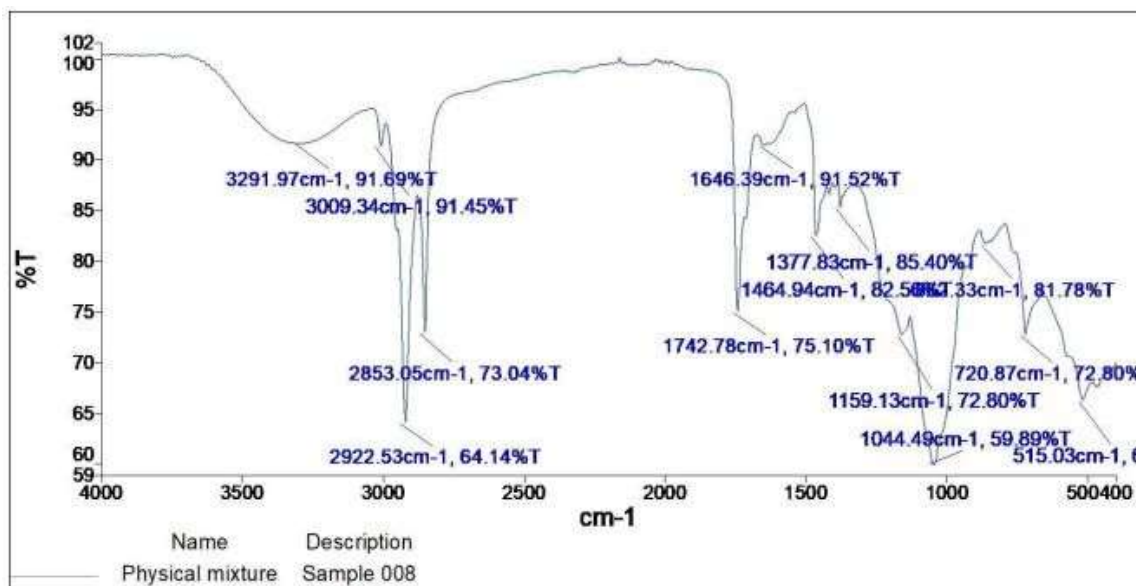


Figure 6.4: FTIR spectrum of Phospholipid (Soya Lecithin).

Table 6.5: FTIR interpretation of Phospholipid (Soya Lecithin).

Reported peak (cm ⁻¹)	Observed peak (cm ⁻¹)	Functional group
2854.96	2853.26	C–H stretching band
1736	1742.75	Carbonyl stretching band
1236.37	1166.82	P=O stretching band

**Figure 6.5: FTIR spectrum of Physical mixture.**

(Bobgunniamadagascariensis extract and phospholipid)

Table 6.6: Interpretation of FTIR spectrum of Physical mixture (Bobgunnia madagascariensis extract and phospholipid).

Reported peak (cm ⁻¹)	Observed peak (cm ⁻¹)	Functional group
2854.96	2853.05	C–H stretching band
1703.54	1742.78	carbonyl C = O stretching
1021.80	1044.49	O-H group
767.71	720.87	C-F group

In vitro drug release tests were performed to examine the release properties of the BM extract-loaded complex phytosome compared to a control product. The research showed that the phytosome mixture greatly slowed the drug release, with $89.460 \pm 0.561\%$ of the medicine released over 12 hours, compared to the control, which released $99.137 \pm 0.510\%$ after 2 hours. This continuous release profile is suggestive of the formulation's potential to improve the bioavailability of the BM extract, providing a more controlled and extended beneficial effect.^[14]

Overall, the results of this study show that the BM extract-loaded complex phytosome product offers great benefits in terms of solubility, drug trapping, stability, and delayed drug

release, making it a good choice for future development in medicinal uses.

DISCUSSION

Pre-formulation Studies

The preformulation tests done in this study were important for defining the basic physical and chemical aspects of the *Bobgunniamadagascariensis* material, which would later impact the design of the phytosome product.^[15] The organoleptic examination, which involved studying the color, taste, and texture, showed that the extract could be properly blended into a product without substantial problems relating to sense qualities. This first step was vital for ensuring that the end product would be accepted by potential customers.

UV spectroscopy study found an absorption maximum at 282 nm, showing the particular range at which the extract shows the largest absorbance. This finding was important for building a calibration curve, which later allowed accurate measurement of the active components in the extract.^[16] The excellent uniformity seen in the calibration curve, with an R^2 value of 0.999, stresses the dependability of the UV spectroscopic method utilized in this study.

Phytosome Formulation and Evaluation

The discovery and review of the BM extract-loaded complex phytosome mixture were important to this research. The high percentage return (94.195% to 96.165%) across various formulas shows the speed of the preparation method. The visual look of the phytosomal complexes was constant, producing a thin layer, which is a positive sign of uniformity and stability in the formation process.^[17]

The solubility tests done in both water and n-octanol showed a large improvement in the solubility of the phytosomal complex compared to the pure extract. This boost in solubility is a major component in improving absorption, since the low solubility of plant products is a well-known limit in drug administration. The better solubility in DCM (13% gain) further supports the promise of phytosome technology to solve the solubility problems associated with standard plant extracts.

Drug Entrapment Efficiency

The drug capture rate, which ranged from 69.422% to 94.621%, is another important finding of this study. The highest trapping efficiency found in formulation F7 shows that the lipid

content plays a key role in the ability of the phytosome to contain the active components successfully.^[18] This high degree of capture is crucial for ensuring that enough of the active component is taken to the target area, therefore increasing the healing potential of the product.

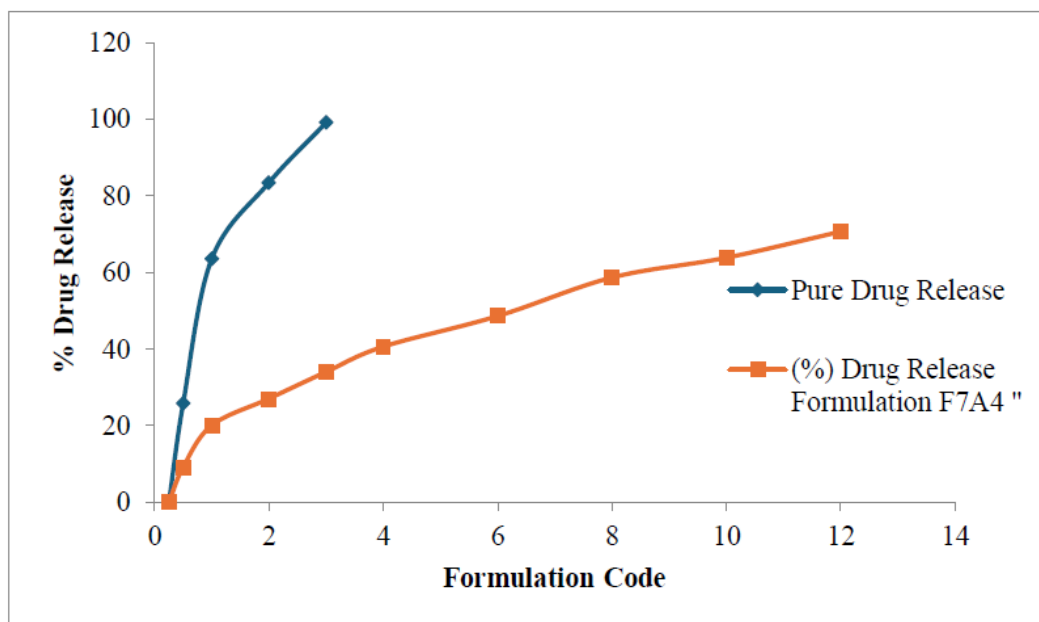


Figure 6.7: Percentage drug release of BM extract loaded complex phytosome&control formulation.

Characterization of the Optimized Formulation

The better version, F7A4, received a thorough evaluation, including particle size distribution, zeta potential, and TEM inspection. The particle size of 252.4 nm and a polydispersity index (PDI) of 0.234 suggests a uniform and stable composition, which is important for consistent drug application and efficiency.^[19] The zeta potential of -29.7 mV shows strong stability, since particles with high zeta potential are less likely to join, which may otherwise affect the formulation's effectiveness.

The TEM pictures showed the cylindrical shape of the phytosomes, which is indicative of well-prepared nanoparticle formulas. This form is useful for drug treatment since it supports better touch with cellular membranes, possibly improving the absorption and solubility of the enclosed extract.

FTIR Analysis

The FTIR study showed insights into the interactions between the Bobgunnia

madagascariensis material, and the phospholipid applied in the preparation. While certain usual peaks of the extract were preserved, small changes showed that complexation had happened, changing the chemical surroundings of the extract.^[20] This interaction is important as it implies that the phytosome mixture effectively holds the active components without sacrificing their integrity, thereby preserving their medicinal potential.

In-Vitro Drug Release Studies

The in-vitro drug release studies showed a considerably longer release of the active components from the phytosome mixture compared to the control. The constant release found in the phytosomal version, with 89.460% of the medicine released over 12 hours, compares starkly with the quick release from the control, which released 99.137% within 2 hours. This constant release is especially beneficial for keeping effective drug levels over a longer time, reducing the need for frequent doses and increasing patient compliance.^[21] The extended-release also suggests that the phytosome preparation might be useful in situations needing long-term medicine treatment, perhaps improving the total therapy results by ensuring more constant drug exposure.

Comparison with Previous Studies

The results of this study align with earlier research on phytosome technology, which frequently shows that phytosomes boost the absorption and medicinal efficiency of plant products. Similar studies have revealed improved drug trapping efficiency, greater stability, and longer drug release patterns when utilizing phytosome formulas.

For instance, the usage of phytosomes has been found to greatly boost the bioavailability of silymarin, a well-known hepatoprotective drug made from milk thistle, by increasing its absorption and lengthening its stay in circulation.^[22] This study's results, which showed improved solubility and prolonged drug release, further support the promise of phytosomes to solve the absorption problems often associated with plant products.

Implications and Future Directions

The successful creation and analysis of the BM extract-loaded complex phytosome mixture have major importance for the area of plant medicine and drug delivery. The improved bioavailability, stability, and delayed release profile of the phytosome mixture suggest that this method might be applied to other plant products having similar problems in solubility and absorption.

Future studies might examine the scale of this preparation method for industrial manufacturing, as well as its usefulness in hospital situations.^[23] Additionally, future research might study the possibility of employing phytosomes to move other troublesome plant products, thereby widening the application of this technology in the pharmaceutical business.

In conclusion, this work provides strong proof that phytosome technology offers a feasible moption for boosting the absorption and therapeutic efficiency of plant products, with substantial potential for improving the treatment of numerous health conditions.^[24] The results encourage the further study and creation of phytosome-based products in both research and industrial medicine uses.

CONCLUSION

The research done in this study has successfully proved the promise of phytosome technology to improve the bioavailability and treatment efficiency of *Bobgunniaadagascariensis* extract, a plant known for its medical qualities but limited by low bioavailability. Through several carefully planned tests, the study addressed major problems related to the delivery of plant extracts, including their low solubility and safety in standard medicine formulas. The preformulation experiments gave a strong basis for the creation of a phytosome-based product, confirming the extract's physical and chemical traits and its interaction with the specified excipients. The use of UV spectroscopy to find the absorption peaks at 282 nm, together with the successful establishment of a valid calibration curve, guaranteed the exact measurement of the extract throughout the study.

One of the most important results was the large rise in solubility found in the phytosomemixture compared to the pure extract. This rise in solubility, especially in both water and n-octanol, is important since it directly aligns with the chance for higher bioavailability. The increased solubility found in the phytosome complex shows that this preparation approach may successfully beat one of the basic limits of plant products in drug administration.

The study also highlighted the amazing drug capture efficiency achieved in the phytosome mixture, with values running up to 94.621%. This degree of effectiveness is indicative of the formulation's ability to contain and spread a considerable amount of the active ingredient, hence improving its medicinal potential. The evaluation of the better formulation further confirmed its suitability, with particle size, zeta potential, and TEM analysis all showing a

stable and uniform formulation that is expected to work well in biological systems. The prolonged drug release described in the in-vitro studies marks another important finding of this work. The phytosome formulation showed a controlled and steady release of the extract over 12 hours, which differs starkly from the fast release from the control formulation. This extended-release feature is especially beneficial for therapeutic uses, since it may keep effective drug levels over a longer time, thus lowering the frequency of dose and improving patient compliance.

The results of this study are comparable with previous research on phytosome technology, which has proven similar improvements in the absorption and efficiency of other plant products. The successful use of this method in *Bobgunniamadagascariensis* extract shows that phytosome-based products might be widely applicable to various herbal medicines, providing a useful answer to the problems of delivering badly soluble plant chemicals. In conclusion, our work has given convincing proof that phytosome technology offers a possible way to improve the solubility and medicinal effectiveness of plant products. The improved solubility, high drug-trapping efficiency, and extended-release profile developed in this study highlight the promise of phytosome products to change the delivery of plant medicines, opening the way for more effective and reliable natural treatments in the future.

Further study and development in this area might lead to significant changes in the field of plant medicine, bringing new and better treatment choices for a broad range of health issues.

REFERENCES

1. Weng, J., Tong, H.H. and Chow, S.F., 2020. In vitro release study of the polymeric drug nanoparticles: development and validation of a novel method. *Pharmaceutics*, 12(8): 732.
2. Fan, Y., Marioli, M. and Zhang, K., 2021. Analytical characterization of liposomes and other lipid nanoparticles for drug delivery. *Journal of pharmaceutical and biomedical analysis*, 192: 113642.
3. Husni, P., Shin, Y., Jeon, H., Lee, E.S., Youn, Y.S., Poon, C.D., Lim, C. and Oh, K.T., 2023. Development and characterization of pH-responsive nanocarriers for chemophotothermal combination therapy of acidic tumors. *Journal of Controlled Release*, 359: 52-68.
4. Yu, H., Alkhamis, O., Canoura, J., Liu, Y. and Xiao, Y., 2021. Advances and challenges in small-molecule DNA aptamer isolation, characterization, and sensor development.

- Angewandte Chemie International Edition*, 60(31): 16800-16823.
5. Clogston, J.D., Crist, R.M., Dobrovolskaia, M.A. and Stern, S.T. eds., 2024. *Characterization of nanoparticles intended for drug delivery* (pp. 63-70). Humana Press.
 6. Cano, E.J., Caflisch, K.M., Bollyky, P.L., Van Belleghem, J.D., Patel, R., Fackler, J., Brownstein, M.J., Horne, B.A., Biswas, B., Henry, M. and Malagon, F., 2021. Phage therapy for limb-threatening prosthetic knee *Klebsiella pneumoniae* infection: case report and in vitro characterization of anti-biofilm activity. *Clinical Infectious Diseases*, 73(1): e144-e151.
 7. Ferro, C., Florindo, H.F. and Santos, H.A., 2021. Selenium nanoparticles for biomedical applications: from development and characterization to therapeutics. *Advanced healthcare materials*, 10(16): 2100598.
 8. Vigata, M., Meinert, C., Huttmacher, D.W. and Bock, N., 2020. Hydrogels as drug delivery systems: A review of current characterization and evaluation techniques. *Pharmaceutics*, 12(12): 1188.
 9. Moris, N., Anlas, K., van den Brink, S.C., Alemany, A., Schröder, J., Ghimire, S., Balayo, T., van Oudenaarden, A. and Martinez Arias, A., 2020. An in vitro model of early anteroposterior organization during human development. *Nature*, 582(7812): 410-415.
 10. Kolla, L., Kelly, M.C., Mann, Z.F., Anaya-Rocha, A., Ellis, K., Lemons, A., Palermo, A.T., So, K.S., Mays, J.C., Orvis, J. and Burns, J.C., 2020. Characterization of the development of the mouse cochlear epithelium at the single cell level. *Nature communications*, 11(1): 2389.
 11. Bahadoran, M., Shamloo, A. and Nokoorani, Y.D., 2020. Development of a polyvinyl alcohol/sodium alginate hydrogel-based scaffold incorporating bFGF-encapsulated microspheres for accelerated wound healing. *Scientific reports*, 10(1): 7342.
 12. Guzmán-Soto, I., McTiernan, C., Gonzalez-Gomez, M., Ross, A., Gupta, K., Suuronen, E.J., Mah, T.F., Griffith, M. and Alarcon, E.I., 2021. Mimicking biofilm formation and development: Recent progress in in vitro and in vivo biofilm models. *Iscience*, 24(5).
 13. Bos, R., Rutten, L., van der Lubbe, J.E., Bakkers, M.J., Hardenberg, G., Wegmann, F., Zuijdgeest, D., de Wilde, A.H., Koornneef, A., Verwilligen, A. and van Manen, D., 2020. Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS- CoV-2 Spike immunogen induces potent humoral and cellular immune responses. *npj Vaccines*, 5(1): 91.
 14. Diaz-Cuadros, M., Wagner, D.E., Budjan, C., Hubaud, A., Tarazona, O.A., Donnelly, S., Michaut, A., Al Tanoury, Z., Yoshioka-Kobayashi, K., Niino, Y. and Kageyama, R.,

2020. In vitro characterization of the human segmentation clock. *Nature*, 580(7801): 113-118.
15. Zakerikhoob, M., Abbasi, S., Yousefi, G., Mokhtari, M. and Noorbakhsh, M.S., 2021. Curcumin-incorporated crosslinked sodium alginate-g-poly (N-isopropyl acrylamide) thermo-responsive hydrogel as an in-situ forming injectable dressing for wound healing: In vitro characterization and in vivo evaluation. *Carbohydrate Polymers*, 271: 118434.
16. Judge, S.J., Murphy, W.J. and Canter, R.J., 2020. Characterizing the dysfunctional NK cell: assessing the clinical relevance of exhaustion, anergy, and senescence. *Frontiers in cellular and infection microbiology*, 10: 49.
17. Soubhagya, A.S., Moorthi, A. and Prabakaran, M., 2020. Preparation and characterization of chitosan/pectin/ZnO porous films for wound healing. *International journal of biological macromolecules*, 157: 135-145.
18. Liu, X., Tan, J.P., Schröder, J., Aberkane, A., Ouyang, J.F., Mohenska, M., Lim, S.M., Sun, Y.B., Chen, J., Sun, G. and Zhou, Y., 2021. Modelling human blastocysts by reprogramming fibroblasts into iBlastoids. *Nature*, 591(7851): 627-632.
19. González-Gualda, E., Baker, A.G., Fruk, L. and Muñoz-Espín, D., 2021. A guide to assessing cellular senescence in vitro and in vivo. *The FEBS journal*, 288(1): 56-80.
20. Rebecca, V.W., Somasundaram, R. and Herlyn, M., 2020. Pre-clinical modeling of cutaneous melanoma. *Nature communications*, 11(1): 2858.
21. Mostafa, D.A.E., Khalifa, M.K. and Gad, S.S., 2020. Zolmitriptan Brain targeting via intranasal route using solid lipid nanoparticles for migraine therapy: Formulation, Characterization, in-vitro and In-vivo Assessment.
22. Tehrani, F., Teymourian, H., Wuerstle, B., Kavner, J., Patel, R., Furmidge, A., Aghavali, R., Hosseini-Toudeshki, H., Brown, C., Zhang, F. and Mahato, K., 2022. An integrated wearable microneedle array for the continuous monitoring of multiple biomarkers in interstitial fluid. *Nature Biomedical Engineering*, 6(11): 1214-1224.
23. Slanzi, A., Iannoto, G., Rossi, B., Zenaro, E. and Constantin, G., 2020. In vitro models of neurodegenerative diseases. *Frontiers in cell and developmental biology*, 8: 328.
24. Jiang, C., Wang, G., Hein, R., Liu, N., Luo, X. and Davis, J.J., 2020. Antifouling strategies for selective in vitro and in vivo sensing. *Chemical reviews*, 120(8): 3852-3889.
25. Chiuppesi, F., Salazar, M.D.A., Contreras, H., Nguyen, V.H., Martinez, J., Park, Y., Nguyen, J., Kha, M., Iniguez, A., Zhou, Q. and Kaltcheva, T., 2020. Development of a multi-antigenic SARS-CoV-2 vaccine candidate using a synthetic poxvirus platform. *Nature communications*, 11(1): 6121.

26. Zielińska, A., Carreiró, F., Oliveira, A.M., Neves, A., Pires, B., Venkatesh, D.N., Durazzo, A., Lucarini, M., Eder, P., Silva, A.M. and Santini, A., 2020. Polymeric nanoparticles: production, characterization, toxicology and ecotoxicology. *Molecules*, 25(16): 3731.
27. Ashammakhi, N., Nasiri, R., De Barros, N.R., Tebon, P., Thakor, J., Goudie, M., Shamloo, A., Martin, M.G. and Khademhosseini, A., 2020. Gut-on-a-chip: Current progress and future opportunities. *Biomaterials*, 255: 120196.
28. Makowski, E.K., Wu, L., Gupta, P. and Tessier, P.M., 2021, January. Discovery-stage identification of drug-like antibodies using emerging experimental and computational methods. In *MAbs* (Vol. 13, No. 1, p. 1895540). Taylor & Francis.
29. Passaro, A.P. and Stice, S.L., 2021. Electrophysiological analysis of brain organoids: current approaches and advancements. *Frontiers in Neuroscience*, 14: 622137.
30. Jayappa, M.D., Ramaiah, C.K., Kumar, M.A.P., Suresh, D., Prabhu, A., Devasya, R.P. and Sheikh, S., 2020. Green synthesis of zinc oxide nanoparticles from the leaf, stem and in vitro grown callus of *Mussaenda frondosa* L.: characterization and their applications. *Applied nanoscience*, 10: 3057-3074.