Research Article

International Journal of Indigenous Herbs and Drugs
Content Available at www.saap.org.in ISSN: 2456-7345

DEVELOPMENT AND CHARACTERIZATION OF PHYTOSOMES CONTAINING *AEGLE MARMELOS* (BAEL)

Rajendra, Kapil Kumar, Aparna Joshi, Anjali Negi, Vaishali Rajput
Division of Pharmaceutics, Global Institute of Pharmaceutical Education and Research, Kashipur, Uttarakhand

Article Info
Article History
Received on: 15-08-2023
Revised on: 27-09-2023
Accepted on: 09-10-2023

Abstract

Aegle marmelos, commonly known as Bael, is a medicinal plant with a wide range of pharmacological properties. However, its therapeutic potential is often limited by its poor bioavailability due to low water solubility and inefficient absorption. Phytosomes, a novel drug delivery system, have emerged as a promising approach to improving the bioavailability and efficacy of herbal extracts. In this study, we aimed to develop and characterize phytosomes containing *Aegle marmelos* extract for enhanced therapeutic benefits. The phytosomes were prepared using a solvent evaporation technique, where phospholipids were complexed with the *Aegle marmelos* extract. The formulation was evaluated using various ratios of phospholipids and extract to achieve maximum entrapment efficiency and drug-loading capacity. The resulting phytosomes were then characterized for their physicochemical properties, including particle size, zeta potential, and morphology using techniques such as dynamic light scattering and transmission electron microscopy. Furthermore, the *in vitro* release profile of the *Aegle marmelos* phytosomes was evaluated using a dissolution apparatus to determine their sustained release behavior. The improved dissolution profile indicated prolonged drug release, suggesting enhanced bioavailability and potential for reduced dosing frequency. In conclusion, our study successfully developed and characterized phytosomes containing *Aegle marmelos* extract, showing improved bioavailability and enhanced cellular uptake. These findings indicate the potential of phytosomes as a promising drug delivery system to optimize the therapeutic benefits of *Aegle marmelos* and potentially other herbal extracts.

Keywords: *Aegle marmelos*, Phytosomes, extract, evaluation.

***Corresponding Author**
Vaishali Rajput

DOI: https://doi.org/10.46956/ijihd.v8i5.489

Production and Hosted By
www.saap.org.in

Introduction

Herbal products have gained immense attention and access to the medicine markets throughout the globe as safer and more effective substitutes for modern synthetic medicines which are considered to be full of adverse and toxic interactions. In underdeveloped and developing nations all over the world plant drugs in traditional forms have been supposed to satisfy the primary healthcare needs of about 80% of the population and even in developed nations these medicines are being utilized by about 65% of the population [1,2]. World Health Organization (WHO) estimates that 80% of the world populations presently use herbal medicine for primary health care. Every nation is seeking health care beyond the traditional boundaries of modern medicine; turning to self medication in the form of herbal remedies. 1 Modern herbal medicine is based upon the combination of traditional knowledge, clinical experience, understanding of medicinal science and scientific evidence of herbal medicine6. Novel herbal drug delivery system opens new way for delivery of herbal drugs at right place, at right concentration, for right period of time and also gives scientific evidence to verify the standardization of herbal drug. Phytosomes are vesicular drug delivery systems that incorporate plant extracts or water-soluble phytoconstituents into phospholipids to produce lipid-compatible molecular complexes. They provide better absorption and bioavailability than the conventional herbal extracts.

SOUTH ASIAN ACADEMIC PUBLICATIONS

Aegle marmelos(L.) Correa (A. marmelos), commonly known as Bael belonging to the family Rutaceae, has been widely used in indigenous systems of Indian medicine due to its variousmedicinal properties [3, 4]. *A. marmelos* has been reported to contain several phytoconstituents mainly marmenol, marmin, marmelosin, marmelide, psoralen, alloimperatorin, rutaretin, scopoletin, aegelin, marmelin, fagarine, anhydromarmelin, limonene, â-phellandrene, betulinic acid, marmesin, imperatorin, marmelosin, luvangentin and auroptene [5]. The aim of the present study was to prepare and evaluate topical phytosomal gel of *Aegle marmelos* with an objective to increase its bioavailability and therapeutic efficacy.

Material and method

Plant Agele marmelos fruits were collected from Kashipur Udham Singh Nagar the plant was selected on thebasis of chemical composition and traditional usage. Soya lecithin HiMedia, Dichloromethane,Ethanol, Hydrochloric acid, Carbopol 934, Triethanolamine, and Propyl Paraben purchased fromSweta Scientific Lucknow.

Preparation of plant extract

The leaves of *Aegle marmelos*were air-dried until dry at room temperature and under shade. The dried leaves were then powdered to a fine grade by using a laboratory scale mill. Further, it was sequentially extracted successively with ethanol using the soxhlet apparatus. The solvent was removed and concentrated in a rotary evaporator and water bath. The dried extracts were stored in the refrigerator for further studies6.

Formulation of phytosomes of *Aegle marmelos*extract byantisolvent precipitation technique

To prepare the phytosomes of *Aegle marmelos* extract, drug extract, and soya lecithin at molar ratio of 1:1, 1:2 ,1:3, 1:4, 1:5, 1:6 and 1:7 were taken in the flask of vacuum rotary evaporator. Dichloromethane was added to the flask. The mixture was shaken at a temperature not exceeding 40˚C for 2 hours. The resultant solution was evaporated by increasing the temperature upto 60˚C and by using a vacuum pump in a vacuum rotary evaporator. Ethanol was added to the flaskwith continuous stirring. The phytosomes were precipitated and ethanol was evaporated undervacuum to remove the traces of solvent. The dried residues were gathered and placed in desiccators overnight then crushed in the mortar and sieved through 80 mesh then subjected to furthercharacterization [7].

Formulation of Gels of Phytosome Complex

Preparation of gel

Gel bases were prepared by separately dispersing Carbopol 934 in distilled water with constant stirring at a moderate speed using a mechanical shaker. The pH of all the formulations was adjusted to 5.5 - 6.5 using triethanolamine [8].

Incorporation of Phytosomal complex into the gel

The solution of phytosomescomplex was prepared in 0.1 ml of ethanol in another beaker and was added to the Carbopol base. Different formulations were prepared using varying concentrations of gelling agent. Prepared gels were stored in suitable containers at room temperature for further studies [9].

Evaluation of Phytosomes

Microscopic view

Optical microscopy was used for the characterization of the complex. The complex was suspended in a buffer and a drop was placed on a slide and covered with a cover slip. A microscopic view of the complex was observed at a magnification of 45X10.

Particle size determination

The particle size of the phytosomes was determined by Dynamic Light Scattering (NANO ZS Malvern instrument) and Zeta potential was estimated on the basis of electrophoretic mobility under an electric field. Particle size measurement was performed following 1/100 (v/v) dilution of phytosomal suspension in redistilled water at 25°C. Zeta potential was measured using the same instrument at 25°C following the same dilution in 1 mM NaCl solution11.

Percentage Practical Yield [12]

Percentage practical yield was calculated to know about the percent yield or efficiency of any method, thus helping in the selection of the appropriate method of production12. Phytosomes prepared were collected and weighed to determine practical yield from the following equation:

$$(\%)\ \text{Yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} X100$$

Entrapment efficiency

100 mg of *Aegle marmelos* phytosomal complex were centrifuged at 2000rpm for 30 min using a Remi centrifuge to separate phytosomes from an entrapped drug. The concentration of the free drug as the supernatant was determined by measuring absorbance at 279nm using UV-Visible spectrophotometer13. The percentage of drug entrapment was calculated by using the formula,

Entrapment efficiency (%) = (Total amount of drug) – (amount of free drug) × 100 (Total amount of drug **…….(2)**

Drug content [55]

Phytosomes equivalent to 10 mg of the drug were accurately weighed and taken into a 100 ml volumetric flask. The contents of the flask were dissolved in a small quantity of ethanol and sonicated for 30 minutes. Volume was adjusted to 100 ml with ethanol. The contents of the flask were filtered and drug content was determined spectrophotometrically using a UV spectrophotometerafter appropriate dilutions [14].

Solubility Determination

To determine the change in solubility due to complexation, the apparent solubility of a drug extract and phytosomal complex was determined by adding an excess amount of drug and phytosomes to 6 ml distilled water, 7.4 pH phosphate buffer, and n-octanol in screw-capped vials. The vials were

SOUTH ASIAN ACADEMIC PUBLICATIONS

then shaken at 25ºC for 24 hr in a water bath. After equilibrium had been attained, the saturated solutions obtained were centrifuged to remove the excess drug (15 min,1000 rpm). The supernatant was filtered immediately and rapidly and diluted suitably with the samesolvent to prevent crystallization. The filtered and diluted solutions were then analyzed spectrophotometrically at 279 nm15.

Evaluation of Gels of Phytosome Complex

Homogeneity

All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates [16].

Measurement of pH

The pH of the phytosome gels was measured with the help of a digital pH meter. 0.5 g of phytosome gel was dissolved in 50 ml of distilled water and stored for two hrs. The measurement of pH of each formulation was determined [17].

Drug content

1 g of the prepared gel was mixed with 100ml of suitable solvent. Aliquots of different concentration was prepared by suitable dilutions after filtering the stock solution and absorbance was measured at 279 nm18.

Rheological study

The measurements of the viscosity of prepared gels were carried out with Brookfield Viscometer (spindle type S–96). The readings of each formulation were taken19.

Spreadability

On a glass plate of 10×5cm, 350mg emulgel was taken and another plate of the same size was dropped from a distance of 5cm. After 1 minute the diameter of the circle spread was measured [20].

Extrudability

In the present study, extrudability was determined by measuring the weight (in grams) required to extrude at least 0.5cm gel from the lacquered aluminum collapsible tube in 10 sec [21].

The extrudability was then calculated by using the following equation:

Extrudability = Applied weight to extrude gel from tube (in grams)
Area(in cm2).

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

The *in-vitro* rug release studies were carried out using a modified Franz diffusion (FD) cell. The formulation was applied on the egg membrane which was placed between the donor and receptor compartment of the FD cell. Phosphate buffer pH 5.5 was used as diffusion media. The the temperature of the cell was maintained at 37˚C. The whole assembly was kept on a magnetic stirrer and the solution was stirred continuously using a magnetic bead. One ml of aliquots were withdrawn from the diffusion medium at specific time intervals for 12 hours and the same quantity of fresh, pre-warmed diffusion medium was replaced for the amount withdrawn. The samples withdrawn were analyzed spectrophotometrically at 279 nm and the cumulative % drug release was calculated22.

Drug Release Kinetics [22]

To know the release kinetics, the data obtained from the *in-vitro* release profile was fitted into various models like: Zero-order kinetics, First order kinetics and Higuchi model According to this model, the fraction of drug from the system is proportional to the square root of time [22].

Stability studies

The stability of a drug in a dosage form at different environmental conditions is important because it determines the expiry date of that formulation. Hence, the stability of the prepared formulation was studied. Stability studies were conducted according by storing the gel formulation at 40oC ± 20C, 70% RH ±5% for 45 days. The samples were withdrawn on the initial, 30th & 45th day and analyzed suitably for the physical characteristics, drug content, and cumulative drug release23.

Result and discussion

Table 1: Composition of the *Aegle marmelos*phytosome.

Batch	Ingredients		
	Aegle marmelos: Soya lecithin	**Dichloromethane (ml)**	**Ethanol (ml)**
PS1	1:1	25	8
PS2	1:2	25	8
PS3	1:3	25	8
PS4	1:4	25	8
PS5	1:5	25	8
PS6	1:6	25	8

Table 2: Evaluation parameters of the phytosome formulations.

Batch	% Practical yield	% Entrapment efficiency	% Drug content
PS1	91.45	89.25	90.25
PS2	86.35	86.72	85.38
PS3	84.69	84.38	84.52
PS4	87.52	83.52	87.39
PS5	81.28	88.62	82.25
PS6	83.48	82.24	81.34

Table 3: Gel formulations of the phytosome complex.

Ingredients	Batch					
	PG 1	**PG 2**	**PG 3**	**PG 4**	**PG 5**	**PG 6**
Carbopol (%)	1	1.5	2	2.5	3	3.5
Triethanolamine (ml)	10	10	10	10	10	10
Propyl paraben (%)	0.1	0.1	0.1	0.1	0.1	0.1
Ethanol	1	1	1	1	1	
Distilled water	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.

SOUTH ASIAN ACADEMIC PUBLICATIONS

Table 4: Evaluation of gels of phytosome complex.

Batch	pH	Viscosity (Centipoise)	% Drug content	Spreadability (cm)	Extrudability (gm/cm2)
PG1	5.2	10562	89.36	4.1	14.7
PG2	5.1	10673	88.42	3.5	10.4
PG3	5.4	11284	83.58	3.8	11.5
PG4	5.0	11298	86.39	3.3	14.5
PG5	5.5	12458	84.25	3.4	14.6
PG6	5.6	13624	85.34	3.0	13.8

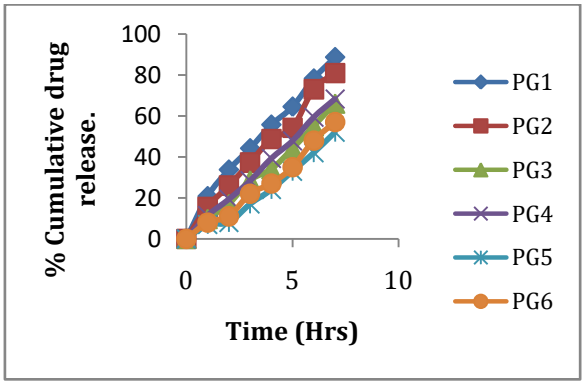

Figure 1: *In-vitro* drug release study of gel formulations.

Table 5: *In-vitro* releases kinetics of different gel formulations.

Batch Code	Zero-order	First order	Higuchi model	Korsmeyer-Peppas model	
	R2	R2	R2	n	R2
PG1	0.933	0.848	0.978	0.695	0.958
PG2	0.921	0.832	0.947	0.712	0.942
PG3	0.913	0.841	0.912	0.673	0.931
PG4	0.926	0.868	0.952	0.721	0.928
PG5	0.922	0.885	0.911	0.684	0.915
PG6	0.932	0.873	0.931	0.731	0.962

Table 6: Stability study of the gel formulation of batch PG1.

S.N.	Parameter	Initial	After 30 Days	After 45 Days
1	Homogeneity	Good	Good	Good
2	Drug content (%)	89.36	89.32	89.31
3	Ph	5.2	5.2	5.2
4	Spreadability (cm)	4.1	4.0	4.0
5	Extrudability (gm/cm2)	9.2	9.2	9.2
6	Viscosity (cps)	10562	10560	10557
7	% Cumulative release	88.55±0.31	88.54±0.09	88.50±0.25

Discussion

Aegle marmelos phytosomes were prepared by the anti-solvent precipitation technique using different ratios of drug and soya lecithin. A total of 6 formulations were prepared. From the prepared formulation the best formulation which contained drug extract: soya lecithin in the ratio 1:1 was selected based on various evaluation parameters and was incorporated into a gel the base of different concentrations using carbopol 934 as a polymer. *All the prepared phytosome of Aegle marmelos were characterized by FT-IR and evaluatedfor % yield, entrapment efficiency, drug loading, particle size, and* **in vitro** *drug release, etc. The evaluation results of Aegle marmelos* phytosome *formulations of (PS1 – PS6).* The formulation of batch PS1 has shown a maximum % yield (91.45) while batch PS5 has shown a minimum practical yield (81.28). Maximum drug content was shown by batch PS1 (90.25), while the minimum is shown by PS6 (81.34). Maximum % Entrapment efficiency was shown by batch PS1 (89.25), while the minimum is shown by PS6 (82.24*) it may be due to the proper bounding of Aegle marmelos with thepolar head of soya lecithin as compared to the others.* The *in vitro* cumulative drug release of phytosome of curcumin (*PS1 – PS6*) is given inTable 7. It showed that the highest was 68.58±0.08% cumulative drug release of PS1 at the endof 7 h. Prepared gel formulations were evaluated on different parameters. The pH of the formulations was in the range of 5.1-5.6. Viscosity was in the range of 10562-13624. Maximum drug content was found in the batch of PG1. Spreadability was in the range of 3.0-4.1 and extrudability was in the range of 10.4-14.7. Stability studies were carried out at accelerated temperatures 400C ± 20C, 70% RH ±5% for 45 days on the gel formulations of PG1. There were no significant changes in the homogeneity, drug content, pH, spreadability, extrudability, viscosity, and *in-vitro* diffusion profile.

Conclusion

The novel drug delivery system research area of herbal drugs is an innovative work that targets for phytoconstituents and plant extracts regarding the therapeutic and cosmetic usefulness of plant products particularly containing flavonoids and polyphenolic compounds. It was observed in this study that *Aegle marmelos* phytosoml gel showed a better diffusion as well as stability profile, hence providing an attractive carrier for the delivery of various phytoconstituents present in it. The present study concludes the successful and efficient

SOUTH ASIAN ACADEMIC PUBLICATIONS

delivery of the *Aegle marmelos* in the form of phytosome gel.

Funding
None

Conflict Of Interest Statement
No conflict of interest.

Ethics Approval and Consent to Participate
Not applicable.

Acknowledgements
None

Reference
1. Bombardelli E, Curri SB, Della RL, Del NP, Tubaro A,Gariboldi P. Complexes between phospholipids andvegetal derivatives of biological interest. Fitoterapia.1989; 60:1-9.
2. Elsaied EH, Dawaba HM, Ibrahim EA, Afouna MI. Investigation of proniosomes gel as a promising carrier for transdermal delivery of Glimepiride. Universal Journal of Pharmaceutical Research 2016; 1(2): 1-10.
3. Hazra, Sudipta Kumar (2020). "Characterization of phytochemicals, minerals and *in vitro* medicinal activities of bael (*Aegle marmelos*L.) pulp and differently dried edible leathers". Heliyon. 6 (10): e05382.
4. Laphookhieo, Surat (2011). "Chemical constituents from Aegle marmelos". J. Braz. Chem. Soc. [online]. 22: 176–178.
5. Rasadah Mat Ali; Zainon Abu Samah; Nik Musaadah Mustapha; Norhara Hussein (2010). *Aegle marmelos* (L.): In ASEAN Herbal and Medicinal Plants (page 107) (PDF). Jakarta, Indonesia: Association of Southeast Asian Nations. p. 43. ISBN 978-979-3496-92-4.
6. Gurpınar SS, Devrim B, Eryılmaz M. In-vitro antibacterial activity of lactobacilli metabolites loaded hydrogel formulations against *Pseudomonas aeruginosa*. Universal Journal of Pharm Research 2019; 4(4):9-11.
7. Hou Z, Li Y, Huang Y, Zhou C, Lin J, Wang Y, Cui F, Zhou S, Jia M, Ye S, Zhang Q. Phytosomes loaded with mitomycin C-Soybean phosphatidylcholine complex developed for drug delivery. Mol. Pharmaceutics. 2013; 10: 90-101.
8. Hamsinah, Meylinda A, Khusnia, Mario R, Ummum A, Safitri D. Application of lutein-zeaxanthin of egg yolk based hydrogel eye mask as a preventive effort against Computer Vision Syndrome (CVS). Universal Journal of Pharmaceutical Research 2021; 6(5):24-29.
9. Singh S, Singh P, Singh S, Trivedi M, Dixit R, Shanker P, "Biological activities and therapeutic potential of *Aegle marmelos*(Bael):a Review", International Research Journal of Pharmaceutical and Applied Sciences, 2013, 3(1);1-11.
10. Türkmen A, Esentürk-Güzel İ, Kara BA. Innovative drug delivery systems for infectious diseases of the skin. Universal Journal of Pharmaceutical Research 2022; 7(4):68-76.
11. Yanyu X,Yunmei S, Zhipeng C, The preparation of silybin-phospholipid complex and the study on its pharmacokinetics in rats, Quineng P Int J Pharm 307(1), 77–82, 2006.
12. Bhattacharya S, Phytosomes: Emerging strategy in the delivery of herbal drugs and nutraceuticals, Pharma Times 41(3), 9–12, 2009.
13. Mursyid AM, Waris R. Formulation and evaluation of pharmaceuticaly stable serum of D. indica (Arbenan). Universal Journal of Pharmaceutical Research 2021; 6(1):38-42.
14. Sahu AR, Bothara SB. Formulation and evaluation of phytosome drug delivery system of *Boswellia serrata* extract. Int J Res Med. 2015; 4(2); 94-99.
15. Paliwal S, Kaur G, Arya KKR. Formulation and characterization of topical nano emulgel of terbinafine. Universal Journal of Pharmaceutical Research 2018;3(6):28-34.
16. Sharma G, Anabousi S, Ehrhardt C, Kumar MNVR J Drug Target 14, 301-309, 2006. 40. Srinivas P, Manthena S, Phytosomes in herbal drug delivery, J Nat Pharm 1(1), 14-18, 2010.
17. Umar S, Onyekachi MK. Development and evaluation of transdermal gel of Lornoxicam. Universal Journal of Pharmaceutical Research 2017; 2(1): 15-18.
18. Vandana SP, Suresh RN, Cardioprotective activity of Ginkgo bilobaphytosomes in isoproterenol-induced myocardial necrosis in rats: A biochemical and histopathological approach, Exp Toxicol Pathol 60, 397–404, 2008.
19. Ugochukwu AE, Nnedimkpa OJ, Rita NO. Preparation and characterization of Tolterodine tartrate proniosomes, Universal Journal of Pharmaceutical Research 2017; 2(2): 1-3.
20. Amit P, Tanwar YS, Rakesh S, Poojan P. Phytosome :Phytolipid Drug Delivery System for Improving Bioavailability of Herbal Drug, *J. Pharm. Sci. Biosci. Res.,* 2013:3(2); 51–
21. Obanewa OA, Oyeniran OT. Development and estimation of anti-inflammatory activity of topical etoricoxib emulgel by carrageenan induced paw oedema method. Universal Journal of Pharmaceutical Research 2019;4(3):22-26.
22. Anyanwu NCJ, Adogo LY, Ajide B. Development and evaluation of in situ gelling gastroretentive formulations of Meloxicam. Universal Journal of Pharmaceutical Research 2017; 2(3): 10-13.