ORIGINAL ARTICLE

Formulation and evaluation of *Cassia auriculata* flower extract-loaded phytosomal cream to enhance the topical bioavailability

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Abstract

Introduction: *Cassia auriculata* flower has various secondary metabolites that are used to treat various diseases, such as bacterial infections. The use of phytophospholipids complexes has been suggested to improve the bioavailability of plant phytoconstituents. Plant active constituent incorporated into phytosome; it efficiently delivers the plant active constituents and provides better absorption for topical administration. **Aim:** This study aims to formulate the phytosomal nanoparticle containing *C. auriculata* flower extract that can increase the topical permeability and absorption of plant compounds. **Materials and Methods:** The phytosomal nanoparticle was prepared by rotary evaporation technique using soy lecithin as a phospholipid and acetone as solvent. The particle size, polydispersity index, zeta potential, entrapment efficiency, *in vitro* release, and antibacterial activity were studied in detail. The phytosome surface morphology was determined by an atomic force microscope. Flower extract-loaded phytosomal dispersion was formulated into cream using beeswax, liquid paraffin, and borax to obtain the cream with a suitable physical appearance and organoleptic characteristics. **Results:** Phytosome was prepared and evaluated. It showed that the particle size of the formulated phytosome was 215 nm. The zeta potential was found to have been −2.67, and the %E.E was found to have been 95 ± 2%. Phytosomal cream was prepared by emulsification technique and its physical properties are analyzed, it was present within the acceptable limits. Ethanol extract of *C. auriculata* flower showed a maximum zone of inhibition of 15.4 ± 0.40 mm against *Staphylococcus aureus*, *Pseudomonas aeruginosa* (12.86 ± 78 mm), and *Escherichia coli* (11.16 ± 0.60 mm). *In vitro* drug release of the formulated phytosome 92.33 ± 1.51 released in phosphate buffer pH 7.4 at the end of 6 h. *Ex vivo* studies were performed on both phytosome-loaded cream and plain extract-loaded cream and compared with plain extract-loaded cream, which shows that phytosome-loaded cream had a better release and topical absorption. **Conclusion:** Studies on phytosome nanoparticles revealed that they were in the nanometer range and could provide the ideal topical absorption of poorly soluble plant active constituents. The phytosome cream showed better percutaneous absorption when compared with conventional plain extract-loaded cream.

Key words: *Cassia auriculata*, flower extract, phytosome cream, flavonoids

INTRODUCTION

Herbal technology is India's one of biggest revenue sources. *Cassia auriculata* is an evergreen plant cultivated in many parts of Tamil Nadu and other parts of India. The fresh and dried flowers, buds, leaves, stems, and roots of *C. auriculata* are used for many diseases, especially in Ayurvedic medicine, used for various conditions such as skin disorders and diabetics. The plant parts of *C. auriculata* show a significant amount of flavonoids, glycosides, saponins, phenols, α-tocopherol-β-D-mannosidase, epicatechin, ferulic acid, quercetin-3-O-rutinoside, tannins, triterpenes carbohydrates, proteins, and amino acids.[2] It belongs to the family Leguminosae. The antimicrobial activity flower part showed the potent antimicrobial activity against the tested pathogens.[3] *C. auriculata* paste was used in joint pain and inflammation

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and is useful in sores on the skin and infections.[4] The main aim of this study is to enhance the percutaneous absorption of plant active constituents and evaluate the antimicrobial properties of prepared cream. Many flavonoids found in *Cassia* species have antiviral and anti-inflammatory, antimicrobial properties. Flavonoids are found in many plants such as lemon, *C. auriculata*. *C. auriculata* flowers were selected based on traditional knowledge and available literature.

MATERIALS AND METHODS

Chemicals for Preparation of Phytosome Nanoparticles

Soy lecithin and quercetin were purchased from Otto Chemie Pvt. Ltd., Mumbai, India. A solvent such as ethanol, acetone, and n-hexane was purchased from Loba Chemie Pvt. Ltd. All other common reagents and solvents are used of laboratory grade.

Excipients for Preparing Cream

Beeswax was purchased from Otto Chemie Private Limited, Mumbai, borax was purchased from HiMedia, and liquid paraffin was purchased from Fisher Scientific-Mumbai.

Identification and Collection of Plant Assets

Fresh *C. auriculata* flowers were collected in Puliangudi, Tenkasi, Tamil Nadu, India, during the month of September 2020–November 2020. The plant model was identified and certified by Drs. C. Murugan, Scientist "E," Botanical Survey of India (BSI) – Coimbatore, reference number BSI/SRC/May/23/2021/Tech/186. The flower parts of the collected plants are dried in the shade and stored for extraction along with other studies.

Extraction Process

The flowers were collected and dried neatly in the shade, and powdered. The finely powdered flower parts were stored in a zip lock cover for continuous use.

Preparation of Flower Extract

Flower extract is made with cold maceration and mechanical shaking method using a mechanical shaker for 48 h. The solvent used was 80% ethanol. Approximately 500 g of powder was soaked in 80% ethanol for 72 h and then macerated at room temperature using a mechanical shaker continuously agitated for 72 h and the extract was filtered, and the solvent evaporated under reduced pressure using a rotary flash evaporator; finally, residues are collected, weighed, and stored at room temperature. The percentage yield of *C. auriculata* flower extract was calculated by the following equation.[5,6]

Percentage yield = (Practical yield/Theoretical yield) × 100

Phytochemical Tests

After completion of *C. auriculata* flower extraction process, extract were tested with phytochemical Tests to determine the presence of various active plant elements. The extraction of the plant is evaluated by the presence of alkaloids, flavonoids saponins, tannins, and carbohydrates.[7]

Phytosome Preparation

A certain amount of flower extract and soy lecithin was taken in a 1:1 ratio[8] and dissolved in an aprotic polar solvent, and sonicated for 20 min using a bath sonicator. It uses high-frequency waves to agitate plant materials in a solvent. Organic solvent removal is done using a rotary flash vacuum evaporator (40–50°C). The mixture is taken in a rotating flask followed by stirring for 1 h at temperatures above (45–50°C). A thin film was obtained to which n-hexane was added and stirred continuously, the precipitate was obtained and the prepared phytosomes were stored in an amber-colored bottle and stored in the refrigerator(4–8°C) for further use.[5,9]

Formulation of Phytosome-Loaded Cream

C. auriculata flower extract-loaded phytosomal dispersion was prepared in cream using beeswax, liquid paraffin, and borax which were used to obtain cream with a suitable physiological appearance and organoleptic characteristics. The cream was prepared from 2 g of beeswax and 6 mL liquid paraffin was taken in a china dish and heated in an electrically heated water bath at 70°C. For the preparation of the aqueous phase take 0.1 g of borax, 2 ml of distilled water in another china dish. The china dish is further heated in an electric water bath at 50°C. The aqueous phase was transferred to a china dish containing phytosome-loaded flower extract with a mixture of beeswax and liquid paraffin with constant stirring at room temperature and placed in a closed container for further evaluation studies.[10] The same method was used to prepare the conventional cream containing flower extract cream ingredients mentioned in Table 1.

Table 1: Composition of cream

Ingredients	Quantity
Active ingredients	2%
Beeswax	2 g
Liquid paraffin	6 ml
Borax	0.1 g
Distilled water	2 ml

Characterization of Prepared Phytosome Nanoparticles

Measurement of particle size, zeta potential, and polydispersity index (PDI)

The Malvern instruments were used to measure the average particle size of the phytosome at 25°C. The prepared phytosome was taken in polystyrene cuvette and average particle size was measured. Malvern Zetasizer can able to measure the size range from 0.1 to 10,000 nm. All the particle size measurements were performed on the diluted phytosomal suspension in a low-volume disposable cuvette at 25°C, and readings were measured at a constant angle. Zeta potential was determined by immersing an electrode used for particle size analysis (Malvern Instrument, Malvern, UK). Samples were diluted with water and kept in an electrophoretic cell.[5]

Percentage entrapment efficiency

The number of active plant substances such as flavonoids entrapped within the phytosome was determined by the process of ultracentrifugation. The centrifugation process was performed at 5000 RPM for 30 min. The supernatant was isolated and spectrometric analyzed at 370 nm. The percentage of drug entrapped in phytosome carriers was determined using the following formula.

%EE = (1- [Drug in supernatant liquid after centrifugation/ Total drug added in formulation]) ×100

In Vitro Drug Release Study

Conventional dialysis sac method

Using a dialysis bag, the *in vitro* drug release profiles of flower extract-loaded phytosomal suspension were evaluated. The dialysis bag was loaded with phytosome suspension and placed in a 500 ml beaker containing buffer. The systems were kept at 37°C with constant stirring at 400 RPM. To keep the sink condition, 5 ml of the sample was withdrawn at predetermined time intervals from the beaker middle position and replaced with fresh buffer. An ultraviolet (UV) spectrometer set to 370 nm, was used to analyze the sample.

Characterization of prepared phytosomal cream

The prepared phytosomal cream was characterized using the following parameters.

Physical Evaluation

The physical appearance, color, and of the flower extract-loaded phytosomal cream were evaluated. These characteristics were evaluated by visual inspection.

Morphological Evaluation by Atomic Force Microscopy (AFM)

The AFM is a kind of scanning probe microscope in which a topographical image of the sample surface can be achieved based on the interactions between a tip and a sample surface. A laser beam is focused onto the back of the cantilever. It can be reflected in a 4-quadrant photodiode detector. With the help of this position-sensitive photodiode, the bending of the cantilever can be measured precisely.

Measurement of pH of Cream

A digital pH analyzer was used to evaluate the pH of phytosomal cream. The pH meter was calibrated with standard pH solutions. The pH was determined after 1 *g* of cream was mixed with distilled water with continuous stirring and kept at room temperature for 2 h and pH was measured 3 times and the average value was obtained.[8]

Texture Analysis

One of the most significant quantitative approaches for measuring the mechanical characteristics of cream is texture analysis. Fixture back extrusion cell was used for forward and backward extrusion of sample to examine the hardness, consistency, of the creams, the probe fixed to penetrate the sample containers containing cream at a depth of 25 mm at a rate of 2.0 mm/s. Texture Pro software was used to record the force applied to the probe. The spreadability test was carried out utilizing a conical shape sample container. These measurements were taken while the cone analytical probe was pushed into the cream at a rate of 2 mm/s.

Homogeneity and Viscosity

Visual inspection was used to ensure that all of the phytosome-loaded creams were homogeneous. Their appearance and the presence of any aggregates in the prepared cream were scrutinized. Cream viscosity was determined using a Brookfield viscometer with spindle No. 7 spinning at 50 RPM at room temperature. The dial reading that corresponded was noted. The cream's viscosity was calculated by multiplying the dial reading by the factor specified in the Brooke field viscometer manual.

Determination of drug content

One gram of the phytosome-loaded cream was weighed and mixed with 10 ml of solvent, and it was agitated for 15 min to completely dissolve. After completion of mixing, it was filtered through a Whatman filter and analyzed at 370 nm.[8]

Ex vivo skin permeation study of phytosome-loaded cream and conventional plain extract-loaded cream

The release of the plant's active constituents like flavonoids, from the medicated creams, was studied using an open-end cylinder. For the skin permeation study, open-end glass cylinders (10 cm high, 3.7 cm outer diameter) were used.

A goatskin was bought from a local shop and trimmed the hair and washed with distilled water and soaked in phosphate buffer pH 7.4 for 12 h before use. The phytosomal cream and conventional flower extract creams were weighed approximately and evenly applied on both skin surfaces and it was fixed to one end of both cylinders with the aid of a rubber band. Both cylinders were fixed in a test tube holder and immersed separately in a 500 ml beaker containing phosphate buffer pH 7.4. It was stirred continuously with the help of a magnetic stirrer. Over the course of 6 h, a sample (5 ml) was taken at various intervals and analyzed for drug content at 370 nm. Each time, the volume was withdrawn and replaced with a new phosphate buffer pH 7.4. A UV spectrophotometer set to 370 nm was used to measure the amount of drug released at different time intervals. Finally, compare the Ex vivo release of plant active constituents. Steady-state flux was calculated.[11] A calibration curve [Figure 1] of standard flavonoids such as quercetin was prepared of known concentrations in the appropriate range,[12] to determine the amount of plant active constituent such as flavonoids that penetrated the skin.

Test Microorganism

Escherichia coli, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were used to test *in vitro* antibacterial activity. All the test microorganism stock cultures were bought from PSG Medical College, Microbiology Department, Coimbatore.

Preparation of Inoculums

All organisms have been grown overnight (24 h) at 37°C on nutrient agar and harvested. Active cultures for experiments have been prepared by the way of transferring a loop full of microorganisms from the cultures to 10 ml peptone broth and incubated without agitation for 12 h at 37°C. All three bacterial inoculums were standardized by matching the turbidity of the microorganism suspension to 0.5 MF standard using diluting with fresh peptone broth and adjusted to the concentration of 10^6 CFU/ml.[13]

Agar Well Diffusion Study

Agar well diffusion method was performed to test the antimicrobial activity of *C. auriculata* flower extract. In this method, accurately weighed 38 g of MH agar dissolved in 1000 ml distilled water and heated for a few minutes until become a clear solution, and autoclaved at 121°C for 30 min, and media solution transferred aseptically into a pre-sterilized media plate. A sterile stainless steel borer was used to make the well by drilling holes and agar plates. Each well was approx. 5 mm wide, and the agar cut was removed using a sterile needle. An accurately weighed phytosomal cream was transferred aseptically into a media plate. Gentamicin 10 µg standard discs were used as a control and placed on an agar medium with the help of sterile stainless steel forceps. The inoculated agar plate was placed at incubator 37°C for 24 h. The tests were carried out in triplicate for each microorganism.[13]

RESULTS AND DISCUSSION

The shadow dried flowers of *C. auriculata* were powdered and extracted with 80% ethanol to obtain an ethanolic fraction and the percentage yield was calculated as 22% w/w.[3] Preliminary phytochemical testing was performed on the extract to detect the presence of various chemical groups of plant active constituents [Table 2]. Flavonoids, saponins, tannins, and carbohydrates were found in the flower extract.[7,14] Nowadays, flavonoids have been used to combat various bacterial infections and skin inflammation and are also used for topical anti-aging preparation due to their ethanol extracts of *C. auriculata* flowers showing antioxidant activity based on scavenging of ABTS radical cation and DPPH radical,[12] due to the presence of flavonoids and tannins. Therefore, the antioxidant property may be one of

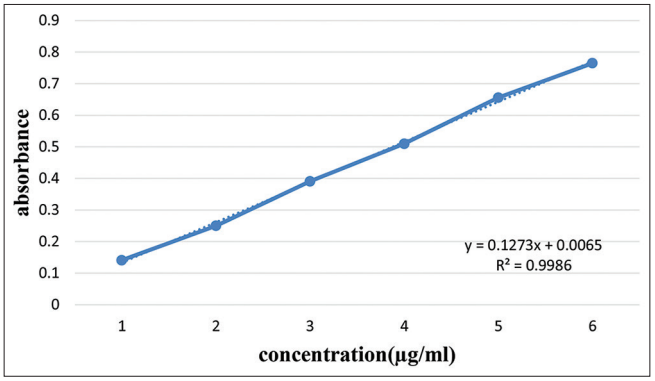

Figure 1: Standard graph of flavonoids

Table 2: Phytochemical screening result of flower extract

Active constituents	Test	Result
Alkaloids	Dragendorff's test	–ve
Flavonoids	Zn-HCl reduction test	+ve
Tannins	Test for tannins	+ve
Saponins	Test for saponin	+ve
Carbohydrate	Molisch's test	+ve
Reducing sugar	Fehling's test	+ve

the mechanisms by which this drug is useful for topical anti-aging preparation and diabetes. There is a lack of research to unambiguously know about the mechanisms involved in a specific pharmacological activity.

Formulation and Evaluation of Phytosome

The phytosome was formulated by solvent evaporation technique using rotavapor. After evaporation of the solvent, the flower extract becomes a thin layer around the rotary flask. The formulation appeared as a reddish-brown color. The nano-sized lipid vesicle was produced by continuous agitation of soy lecithin and along with *C. auriculata* flower extracts at temperature 50–70°C until complete removal of solvent. The prepared phytosomes were collected and stored in an airtight container. The phytosome percentage yield was calculated as 62%.

The particle size and PDI of the prepared phytosome were calculated using the Malvern instrument, and the zeta potential for the formulation was analyzed by the same Malvern instruments (report showed in Figure 2a and b). Before analysis, distilled water was used to dilute the sample. After performing the particle size determination, the average diameter of phytosomal formulation was found to be 215.76 nm and the PDI was 0.216, as shown in Table 3. This nanoscale range is useful for avoiding the problems of low bioavailability and solubility associated with traditional topical herbal drug delivery of plant active constituents.[5]

The electrophoretic mobility of the phytosomal dispersion was analyzed using Malvern Zetasizer and the average zeta potential of flower extract-loaded phytosomal nanoparticles was −2.67. The zeta potential's magnitude reveals the colloidal system's potential stability. The suspension has a strong negative zeta potential, it will repel one another and there is no tendency to come together.

Phytosomes showed a high percent loading of the plant active constituents, which could be an important aspect for therapeutic drug delivery. The ultracentrifugation technique was used to determine the entrapment efficiency of prepared phytosomal nanoparticles. The entrapment efficiency was estimated to be 95 ± 2%.[5]

In vitro drug release was studied for phytosomal nanoparticle dispersion by the conventional dialysis bag method. The formulation that had the high entrapment of drug molecules showed better release. Diffusion of the drug across the dialysis bag happens in two steps. Initial transfer of plant active constituents (encapsulated in phytosome) happens from phytosomal dispersion onto the surface of the dialysis membrane, later, it is moved to the beaker containing buffer solution. A maximum of 92.33 ± 1.51% of the drug was released at the end of the 6th h [Table 4]. In this, a biphasic

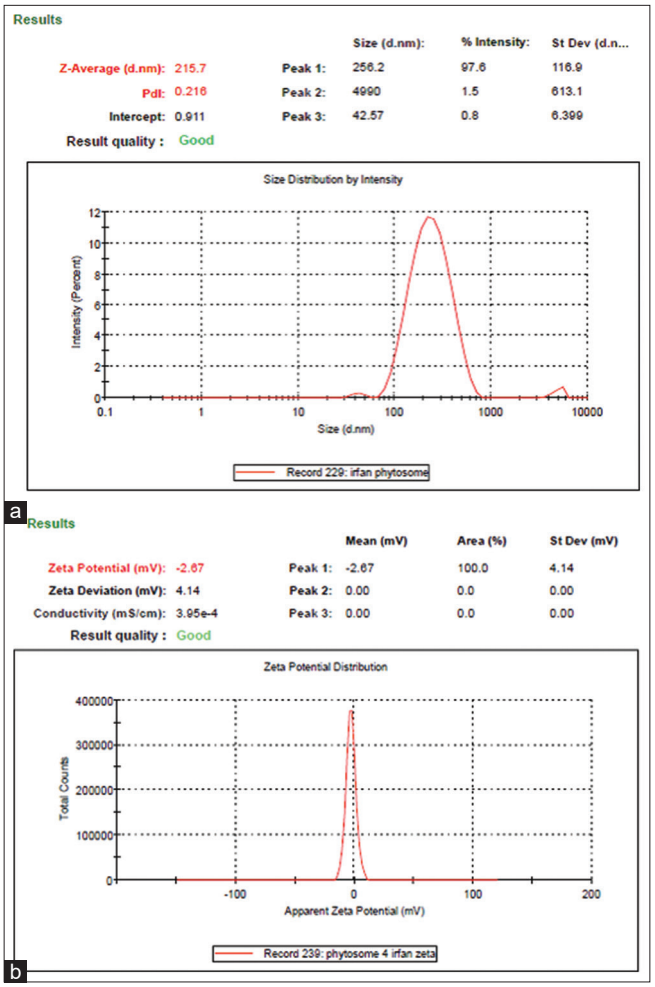

Figure 2: (a) Particle size report of phytosomal nanoparticle, (b) Zeta potential report of phytosomal nanoparticle

Table 3: Evaluation of phytosomal nanoparticle

Formulation	Particle size (nm)	Zeta potential	PDI	% Entrapment
Phytosome	215.76 nm	–2.87	0.216	97%

Table 4: *In vitro* drug release of phytosome

Time (h)	Phytosome percentage released (%)
1 h	16.5±0.2
2 h	20.16±0.76
3 h	40.0±1.5
4 h	56.0±2.23
5 h	73.0±2.0
6 h	92.33±1.51

release pattern was observed. During the 1st h, a burst release happened due to the unentrapped plant active constituents, followed by a sustained release pattern of active constituents from the phytosome [Figure 3]. This is because plant active constituents get highly complexed into the lipid matrix.

Morphological Evaluation by AFM

The AFM cantilever deflects in response to atomic force variations between the tip and the phytosome sample, and the deflection is measured by the detector. The image that was created is a topographical representation of the phytosome sample surface. The morphological evaluation was determined for phytosome with AFM and it is shown in Figure 4.

Formulation Preparation

The physical appearance, color, homogeneity, and consistency of the formulated phytosomal cream were all evaluated. Visual observation was used to assess the color, the appearance of the cream, and homogeneity was tested by pressing a small amount of the formulated cream between the thumb and index finger. The outcomes are listed in Table 5. The color of the prepared cream was light green in color and the appearance of the cram was homogeneous and it was smooth on application. The phytosomal cream did not find any phase separation on storage and was also free from grittiness.

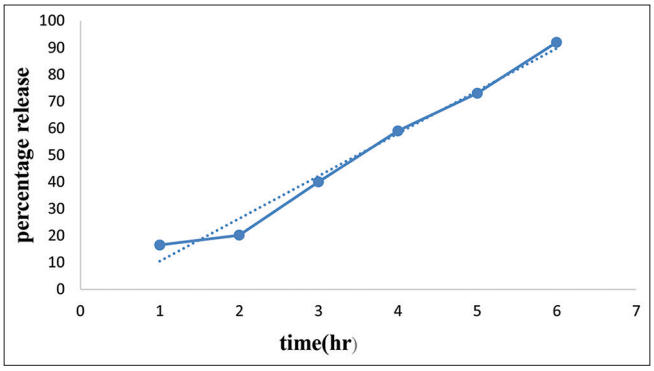

Figure 3: *In vitro* release profile of phytosome

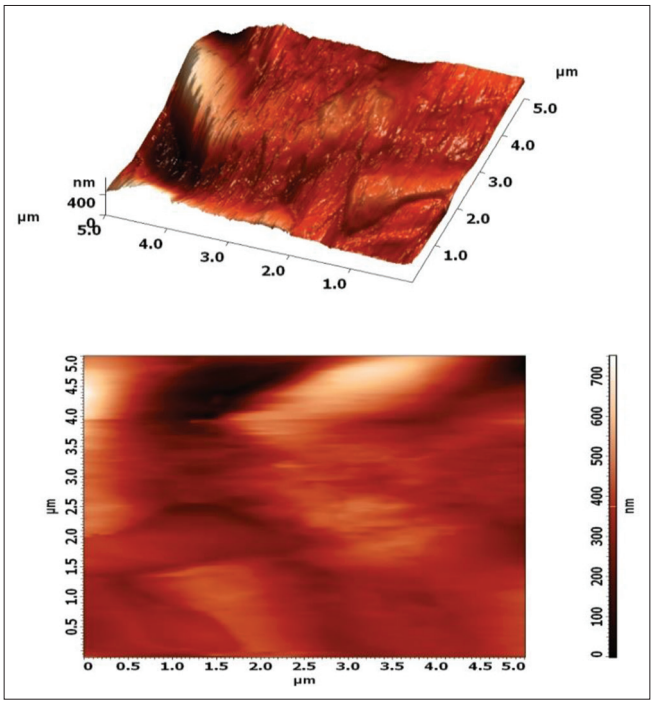

Figure 4: Atomic force microscopic images of phytosome

At room temperature, the pH and viscosity were determined. At room temperature, the pH of the formulated phytosomal cream was tested using a pH meter and determined to be 5.6 ± 0.2 which is ideal to treat skin infection. The viscosity of phytosome-loaded cream ranged from 45 × 10^3 ps. With the help of a texture analyzer, the spreadability, firmness, and extrudability of phytosomal cream were measured. The spreadability of the cream formulation was found to be 2101.402 g.s and firmness was found to be 2066.023 g and the extrudability was 8646.082 g which showed that the formulation should be spread evenly before the absorption occurs. This will also allow better absorption at the site of application. The spreadability and extrudability graphs are shown in Figures 5 and 6 which showed that formulation was evenly spread on the skin by applying a small amount of shear. The percent drug content of flower extract-loaded phytosomal cream was found to be 88 ± 0.72%, which shows that phytosomal dispersion was well incorporated into the cream base.

Ex Vivo Skin Permeation Study of Phytosome-Loaded Cream and Plain Extract-Loaded Cream

A skin permeation test was carried out on goatskin using an open-end cylinder tube with phytosomal and conventional creams, and the estimated drug penetration in the skin was measured for up to 6 h. From the result shown in Figure 7 and Table 6, the greater flux value and permeability coefficient of phytosomal cream indicate that there is a minor increase

Table 5: Evaluation test of plant extract-loaded phytosomal cream

S. No.	Parameter	Result
1.	pH	5.6±0.2
2.	Homogeneity	Good
3.	Color	Light green
4.	Appearance	Uniformity in color
5.	Spreadability	Good spread
6.	Smear type	Non-greasy
7.	Removal	Easy removal

Table 6: Flux and permeability coefficient of the cream

Formulations	Flux (mg/cm²/h)	Permeability coefficient (cm/h)
Phytosomal cream	12.96±0.312	26.03±0.856
Plain extract-loaded cream	13.39±0.141	26.413±0.560

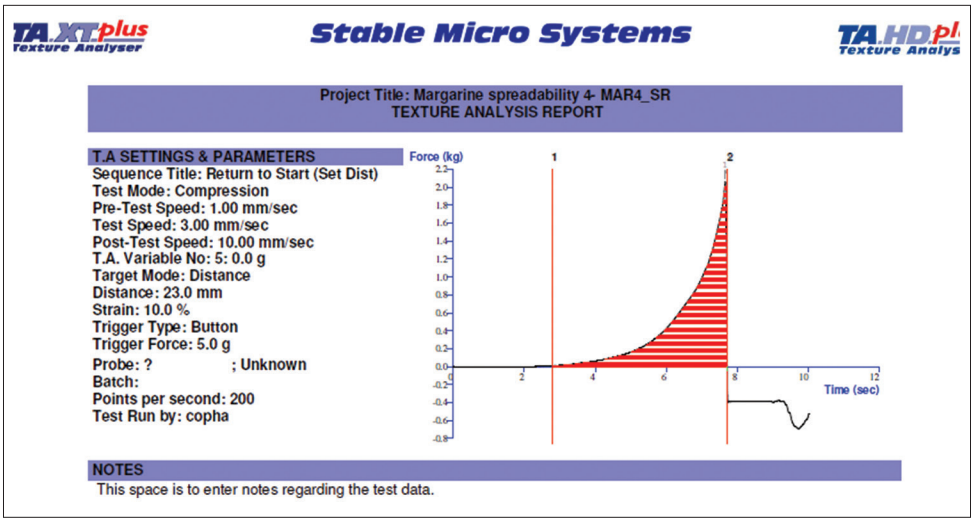

Figure 5: Spreadability report of the cream

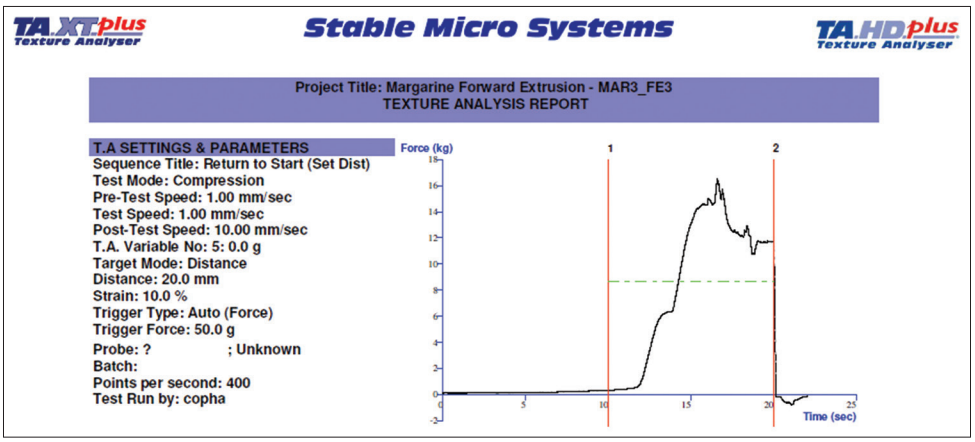

Figure 6: Extrudability report of the cream

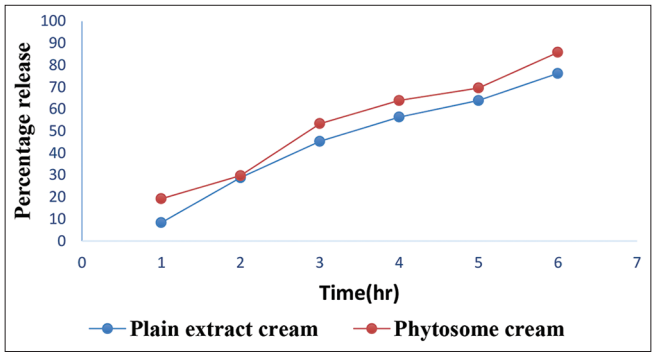

Figure 7: *Ex vivo* release profile of phytosome-loaded cream versus plain extract-loaded cream

in plant active constituents penetration through the skin, and hence increasing the bioavailability of plant active ingredients and improving therapeutic effect.

In Vitro Antibacterial Activity

Agar well diffusion method was performed to test the antimicrobial activity of *C. auriculata* flower extract containing phytosome loaded cream. An ethanolic extract of *C. auriculata* flower exhibits strong antimicrobial activity against all the tested microorganisms.[15] The antibacterial activity of *C. auriculata* flower extract-loaded phytosomal cream was tested using the agar well diffusion technique. The 80% ethanolic extract of *C. auriculata* flower has significant antibacterial activity against all species tested in a concentration-dependent manner with a maximum zone of inhibition of 15.4 ± 0.40 mm against *S. aureus*, *Pseudomonas aeruginosa* (12.86 ± 78 mm), and *E. coli* (11.16 ± 0.60 mm);[2] it is shown in Table 7.

Flavonoids, tannins, saponins, and a variety of other plant active constituents are secondary metabolites of plants that serve as a defense mechanism against a wide range of microorganisms. Flavonoids are hydroxylated phenolic compounds produced by a plant in response to microbial infection. Saponins antimicrobial properties are due to its ability to cause protein and enzyme leakage from cells. Tannins bind to proline-rich proteins, preventing them from being synthesized.[16] The medicinal properties and pharmacological actions of *C. auriculata* are well-known to

Table 7: Antibacterial activity of flower extract-loaded phytosomal cream

S. No.	Tested microorganism	Zone of inhibition of STD (mm)	Zone of inhibition of test (mm)
1	*Staphylococcus aureus*	20.33±0.57	15.4±0.40
2	*Escherichia coli*	17.93±0.16	11.16±0.60
3	*Pseudomonas aeruginosa*	22.33±0.30	12.86±78

Indian traditional medicine. Natural materials have sparked a lot of interest in the past few years as potential sources of new antibacterial agents. Natural resources have attracted a lot of attention as possible sources of novel antibacterial agents in recent years.

CONCLUSION

C. auriculata phytosome was prepared and it was converted into a cream dosage form. Soy lecithin is the most frequently used to prepare phospholipid complexes that are made up of two hydrophobic hydrocarbon chains and a hydrophilic head group. The membrane permeability and oil-water partition coefficient of plant active components are considerably enhanced after generating phospholipid complexes.[1] In comparison to conventional cream, phytophospholipid complexes are more easily absorbed and produce better bioavailability.[9] *Ex vivo* release of the plant active constituents from phytosomal cream was superior to that of the traditional plain extract-loaded cream. Encouragingly, the technique of phytophospholipid complexes has overcome the obstacle of poor bioavailability for many plant active constituents such as flavonoids, saponins, and tannins.[17]

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