



MUMBAI UNIVERSITY



## PROJECT REPORT: FINAL YEAR B. PHARM (2024-25)

# FORMULATION AND EVALUATION OF STAPHYSAGRIA ENRICHED NDDS SHAMPOO

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MUMBAI UNIVERSITY

**PRINCIPAL K. M. KUNDNANI COLLEGE OF PHARMACY  
CUFFE PARADE, MUMBAI – 400005**

**PROJECT WORK REPORT**

**TITLE: FORMULATION AND EVALUATION OF  
STAPHYSAGRIA ENRICHED NDDS SHAMPOO.**

**DEPARTMENT: PHARMACOGNOSY**

**FINAL YEAR B. PHARM R (2019) SEM VIII  
ACADEMIC YEAR 2023-2024**

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**CERTIFICATE**

**THIS IS TO CERTIFY THAT THE FOLLOWING STUDENTS OF FINAL YEAR B. PHARM HAVE ATTENDED AND SUCCESSFULLY COMPLETED PROJECT TITLED: “FORMULATION AND EVALUATION OF STAPHYSAGRIA ENRICHED NDDS SHAMPOO” IN THE ACADEMIC YEAR 2024-25.**

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**IS A BONAFIDE WORK DONE BY THEM  
UNDER MY GUIDANCE**

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**STATEMENT BY THE CANDIDATE**

We wish to state that the work in this project titled “**FORMULATION AND EVALUATION OF STAPHYSAGRIA ENRICHED NDDS SHAMPOO**” forms our own contributions to the project work carried out under the guidance of **MR. PAVAN KUMAR R. SINGH** at Principal K. M. Kundnani College of Pharmacy, Cuffe Parade, Colaba, Mumbai- 400005. This work has not been submitted for any other degree at this or any other University.

**SIGNATURE OF THE CANDIDATES:**

| <b>SR. NO.</b> | <b>Name of Students</b> | <b>Roll. No.</b> | <b>Signature</b> |
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| 1              | <b>Yash Dhanwani</b>    | <b>15</b>        |                  |
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An idea without implementation is merely a passing thought, but with the right guidance and support at the right time, it becomes a reality.

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## 1. Abstract

The growing preference for natural and sustainable personal care products has encouraged the exploration of plant-based alternatives to synthetic formulations, which may cause irritation, allergic reactions, and long-term health risks. This study focuses on the development of an innovative herbal shampoo incorporating *Staphysagria* oil, known for its therapeutic properties, through phytosome technology.

Initially, a preliminary phytochemical analysis of *Staphysagria* oil was conducted, confirming the presence of bioactive potential. To enhance the oil's bioavailability, stability, and efficacy, a phytosome complex was prepared. Phytosomes are advanced delivery systems that encapsulate active compounds, improving their ability to penetrate biological membranes.

The prepared phytosome was incorporated into a shampoo base. The formulation was designed to ensure compatibility, stability, and ease of use while maintaining the integrity of the active components. The anti-bacterial and antifungal activity of the developed shampoo was evaluated using the agar cup plate method, a reliable technique for assessing the inhibition of bacterial and fungal growth. Widely used agents such as Selenium sulfide for anti-bacterial and Ketoconazole for antifungal served as the standard for comparison.

The results revealed that the *Staphysagria* oil-based shampoo exhibited excellent anti-bacterial and antifungal efficacy, outperforming Selenium sulfide and ketoconazole in terms of zone of inhibition. These findings emphasize the potential of this herbal formulation as an effective and safe alternative for managing hair and scalp conditions, such as dandruff, fungal infections, itching and flaking scalp, etc.

This research demonstrates the innovative application of NDDS in herbal formulations, offering a promising avenue for natural product development in the hair care industry.

## **2. Aim and Objectives**

### **Aim**

To develop a Novel Drug Delivery System (NDDS) formulation in the form of a phytosome using *Staphysagria*, and to incorporate this phytosome into a shampoo formulation for enhanced efficacy, and stability, thereby providing a natural and innovative solution for hair and scalp care problems.

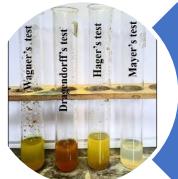
### **Objective**

1. To extract bioactive constituents from *Staphysagria* using an appropriate extraction method.
2. To perform preliminary phytochemical screening of *Staphysagria* extract to identify its bioactive constituents.
3. To develop and optimize a phytosome formulation using *Staphysagria* extract as the active ingredient.
4. To evaluate the phytosome formulation for key parameters such as entrapment efficiency, particle size, zeta potential and stability.
5. To incorporate the prepared phytosome into a shampoo base to create a novel herbal shampoo formulation.
6. To evaluate the formulated shampoo for its antifungal and antibacterial activities in effectively addressing hair and scalp care concerns.

### 3. Plan of work



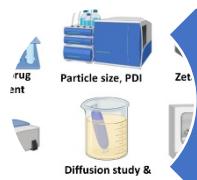
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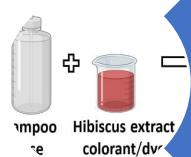
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To incorporate the prepared phytosome into a shampoo base to create a novel herbal shampoo formulation.



To evaluate the formulated shampoo for its antifungal and antibacterial activities for hair and scalp care concerns.



Effective Herbal Shampoo for Scalp & Hair Care

## 4. Introduction

Dandruff is a widespread scalp condition that affects over 50% of the global population, often resulting from microbial infections, particularly *Candida albicans* and *Staphylococcus aureus*, as well as factors like stress, poor diet, and environmental pollution.<sup>[6,7]</sup> While dandruff is generally considered a mild condition, if left untreated, it can progress to seborrheic dermatitis, scalp inflammation, persistent itching, hair thinning, and even hair loss. Additionally, the discomfort and visible flakes associated with dandruff can significantly impact an individual's self-esteem and confidence.<sup>[1,2]</sup>

Traditional anti-dandruff shampoos commonly rely on harsh synthetic ingredients, including sulfates, parabens, and synthetic antifungal agents, which may offer temporary relief but do not effectively target the root cause of dandruff.<sup>[10,17,18]</sup> Prolonged use of these chemical-based formulations often leads to scalp irritation, excessive dryness, and potential long-term hair damage.<sup>[16,22,24]</sup> As a result, there is a growing demand for safer, herbal-based alternatives.<sup>[12,26]</sup> However, many existing herbal formulations fall short due to poor absorption, limited bioavailability, and a lack of comprehensive action, often addressing only one aspect of dandruff, such as fungal overgrowth, without promoting overall scalp health.<sup>[3,11,27]</sup>

*Staphysagria*, a medicinal plant used for centuries in traditional medicine, possesses antifungal, antibacterial, and anti-inflammatory properties, making it a promising natural remedy for dandruff. Despite its therapeutic potential, it remains underutilized in modern anti-dandruff formulations due to poor solubility and inadequate scalp penetration, limiting its effectiveness in conventional shampoos.<sup>[4,5,8]</sup>

To overcome these challenges, our project focuses on the development of a *Staphysagria*-based anti-dandruff shampoo utilizing a Novel Drug Delivery System (NDDS).<sup>[13,20,21]</sup> By incorporating *Staphysagria* into an advanced lipid-based phytosomal system, we significantly enhance its absorption, stability, and bioavailability, ensuring deep scalp penetration and prolonged therapeutic action.<sup>[14,15]</sup> This innovative formulation not only combats dandruff at its source but also provides comprehensive scalp nourishment, reducing irritation, soothing inflammation, and promoting long-term scalp health.<sup>[23,25]</sup>

Through this project, we aim to bridge the gap between traditional herbal wisdom and modern pharmaceutical advancements, offering a natural, effective, and scientifically enhanced alternative to conventional anti-dandruff treatments.<sup>[9,28]</sup>



***Staphysagria* Seed**

**Scientific Name:** *Staphysagriamacrosperma*

**Taxonomical classification:**

|          |                                |
|----------|--------------------------------|
| Kingdom  | Plantae                        |
| Division | Streptophyta                   |
| Class    | Equisetopsida                  |
| Order    | Magnoliidae                    |
| Family   | Ranunculaceae                  |
| Genus    | Staphysagria                   |
| Species  | <i>Staphysagriamacrosperma</i> |

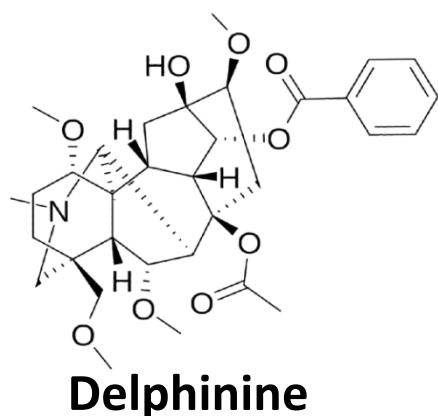
## **Chemical Constituents:**

It includes Delphinine, Staphysagrine, Staphynoline, Staphisagrine, Lycaconitine, Methyllycaconitine, Kamferol, Quercetin.

## **Uses:**

- *Staphysagria* helps in treating skin infections, wounds, and microbial conditions.
- It is used in shampoos to reduce dandruff and soothe scalp irritation.
- It promotes faster healing of cuts, ulcers, and wounds.
- It has been traditionally used to treat lice, scabies, and other parasitic infestations.
- It is used in homeopathy to manage suppressed emotions, grief, and nervous disorders.
- It helps in reducing swelling, redness, and irritation in various skin conditions.
- It is used for relieving nerve pain, headaches, and post-surgical pain.
- It helps in treating burning urination and bladder irritation.

## **Structure of delphinine**



## **5. Experimentation**

### **5.1 Collection of Herb**

The crude drug (seed of *Delphinium Staphysagria* Linn.) was obtained as a gift sample from Dr. Palep's Research lab, Mumbai.

### **5.2 Processing of crude drug:**

The seeds were subjected to drying under controlled shelter conditions and subsequently processed for extraction.

### **5.3 Extraction of Herb**

- Extraction was carried out by Maceration.
- Coarsely powdered drug material (seed) is placed inside a container; the menstruum (ethanol) is poured on top until completely covering the drug material.
- The container is then closed and kept for at least 14 days.
- The content is stirred from time to time to ensure complete extraction.
- At the end of extraction, the menstruum is separated from marc by filtration.
- Concentrated to get dried powder extract.

### **5.4 Phytochemical Screening:**

#### **a. Alkaloids**

##### **i. Mayer's test:**

Principle: Mayer's reagent (potassium mercury iodide solution), a solution of potassium mercury iodide, reacts with alkaloids to form a potassium-alkaloid complex, which precipitates out of solution as a cream-colored precipitate.

Procedure: Extract + few drops of Mayer's reagent.

Creamy white ppt is observed.

##### **ii. Hager's test:**

Principle: Alkaloids, being basic (alkaline) organic compounds that contain nitrogen, react with Hager's reagent (a saturated solution of picric acid, which

is acidic) to form insoluble yellow-colored precipitates due to the formation of alkaloid-picrate complexes.

Procedure: Extract + few drops of Hager's reagent.

Yellow ppt is observed.

**iii. Dragendorff's test:**

Principle: Alkaloids, which are basic nitrogenous compounds, react with Dragendorff's reagent to form an orange or reddish-brown precipitate due to the formation of insoluble ion-association complexes between the positively charged alkaloids and the negatively charged complex ions in the reagent.

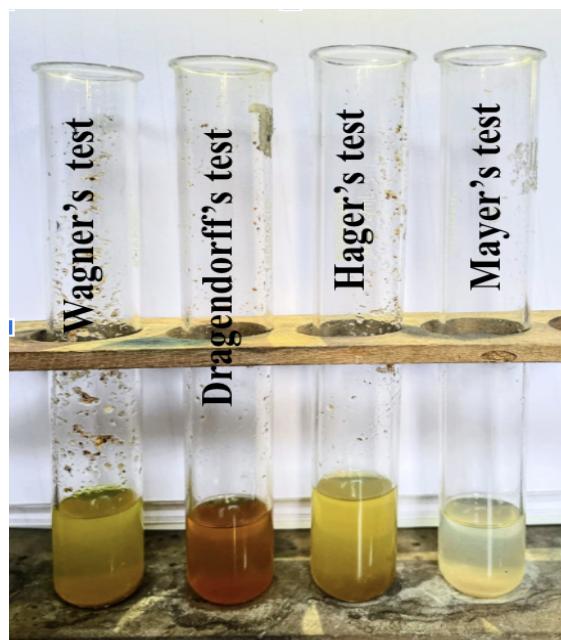
Procedure: Extract + few drops of Dragendorff's reagent.

Orange Reddish ppt is observed.

**iv. Wagner's test:**

Principle: Wagner's reagent (iodine in potassium iodide) reacts with alkaloids to form a reddish-brown precipitate due to the formation of insoluble ion-association complexes between the positively charged alkaloids and polyiodide ions in the reagent.

Procedure: Extract + few drops of Wagner's reagent. Reddish Brown is observed.



| Phytoconstituent | Test  | Observation  | Inference              |
|------------------|---|--|------------------------|
| Alkaloids        | <p>Extract + few drops of Mayer's reagent</p> <p><b>1. Hager's test:</b><br/>Extract + few drops of Hager's reagent</p> <p><b>2. Dragendorff's test:</b><br/>Extract + few drops of Dragendorff's reagent</p> <p><b>3. Wagner's test:</b><br/>Extract + few drops of Wagner's reagent</p> | <p>Creamy white ppt</p> <p>Yellow ppt</p> <p>Orange</p> <p>Reddish ppt</p> <p>Reddish</p> <p>Brown</p> | Alkaloids are present. |
| Glycosides       | <p><b>Keller Kilani test:</b><br/>A solution of 0.5ml, containing glacial acetic acid and 2-3 drops of ferric chloride, was mixed with 2 ml of extract. Later, 1 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added along the walls of the test tube.</p>                           | No Reddish-brown ring was observed at the junction of two liquids.                                     | Glycosides are absent. |

|               |   |  |                           |
|---------------|---|--|---------------------------|
| Carbohydrates | <b>Molisch test</b><br><p>Few drops of alcoholic alpha – naphthol solution were added to 2 ml of extract. Later, few drops of concentrated H<sub>2</sub>SO<sub>4</sub> were added along the walls of the test tube.</p> | No violet ring was observed at the junction. | Carbohydrates are absent. |
| Saponins      | <b>Foam test</b><br><p>5ml of extract was shaken vigorously, then it was left to rest for five minutes.</p>   | No Foam formation                            | Saponins are absent.      |
| Flavonoids    | <b>Shinoda test</b><br><p>10 drops of dilute HCL and a piece of magnesium were added to 1 ml of extract.</p>  | No Deep pink color was observed              | Flavonoids are absent.    |
| Tannins       | <b>Ferric chloride test</b><br><p>Add a few drops of 5%FeCL<sub>3</sub> solution to the extract.</p>  | No Blue-black color was observed             | Tannins are absent.       |

## **5.5 Determination of Total Alkaloidal Content:**

Determination of total alkaloidal content is a quantitative analysis used to estimate the amount of alkaloids present in plant materials. Alkaloids are bioactive compounds with therapeutic properties, commonly found in medicinal plants. This analysis helps in standardizing herbal formulations and ensuring their efficacy. It is widely used in pharmacognosy, phytochemistry, and quality control of herbal products.

### **Procedure:**

- 2gms of *Staphysagria* powder was taken in a conical flask and to it 30ml of Butanol was added. It was extracted by heating up to 30 mins.
- Extract was filtered and volume was made up to 30ml.
- 10 ml of this sample was taken in a conical flask and to this 10ml of HCl was added.
- This mixture was then subjected to separation in a separating funnel.
- The HCl layer was separated out.
- The resultant mixture was then titrated against 0.1N NaOH using methyl red as an indicator.
- End point was from pink to lime yellow.
- Blank was also performed using 10 ml of distilled water + 10 ml HCl + methyl red.



## 5.6 Thin Layer Chromatography (TLC):

- Made extract alkaline with ammonia solution and extract the aqueous layer with chloroform.
- Concentrate the chloroform extract to 2 ml and Co-TLC was carried out with Delphinine using chloroform: methanol (9:1 v/v) as mobile phase and Dragendorff's reagent as a spraying reagent.
- Spot corresponding to Delphinine appears.



## 5.7 Ultraviolet-visible (UV-Vis) spectroscopy:

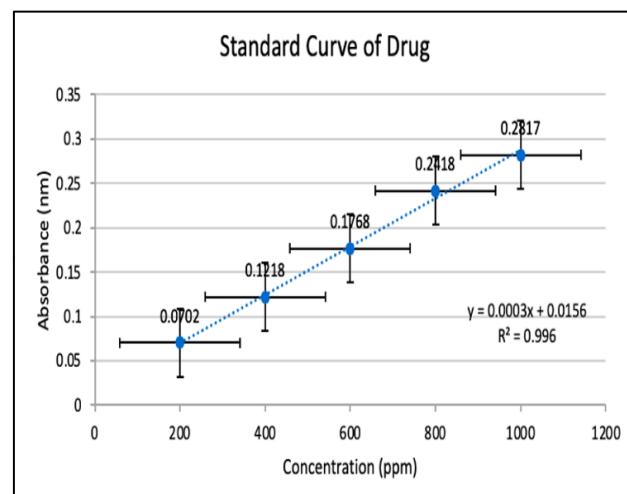
### 5.7 (a) Determination of maximum wavelength ( $\lambda_{\text{max}}$ ) and standardization of extract:

Ultraviolet-visible (UV-Vis) spectroscopy was performed on the *Staphysagria* extract (1000 ppm) and compared with the standard alkaloid, delphinine, to confirm its presence and evaluate its characteristics.

### 5.7 (b) Preparation of standard calibration curve:

A standard curve was prepared by using various concentrations of the standard solution, including 200, 400, 600, 800, and 1000 ppm, to establish a calibration line for quantitative analysis at a fixed wavelength of 270 nm.

| Concentration (ppm) | Absorbance (270 nm) |
|---------------------|---------------------|
| 200                 | 0.0702              |
| 400                 | 0.1218              |
| 600                 | 0.1768              |
| 800                 | 0.2418              |
| 1000                | 0.2817              |



## 5.8 Preparation of Phytosome

Phytosome was prepared by solvent injection technique.

Required amount of alcohol was used to dissolve the extract and 15 ml of phosphate buffer was used to make up volume. This phase was termed as aqueous phase.

In the organic phase, cholesterol, lipid (P-100) and ethanol were added. Both these phases were subjected to ultrasonication for about 20-30 minutes. A stabilizer was added to the aqueous phase in order to maintain its stability. In a beaker the aqueous phase was kept on magnetic stirrer at room temperature and a further organic phase was injected to aqueous phase drop wise.

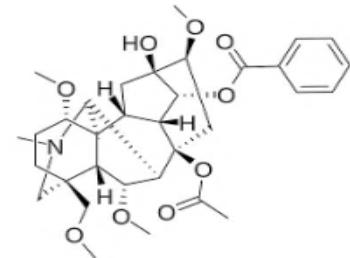


## 5.9 Optimization of Phytosome:

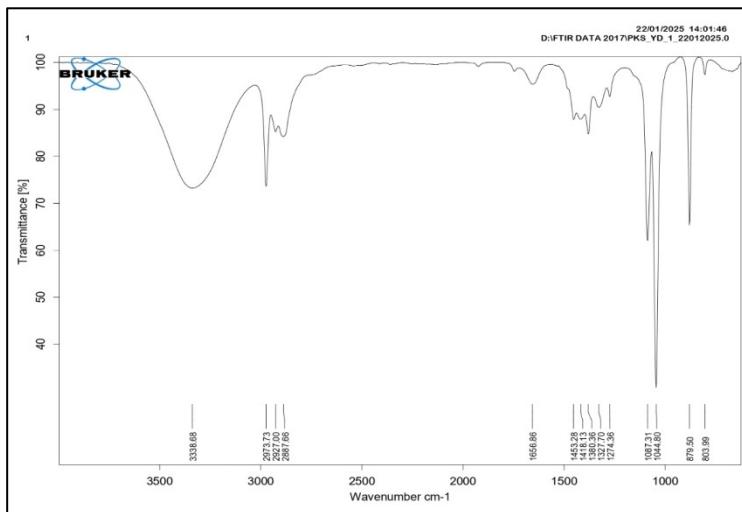
| Batch No. | Extract (mg) | Lipid (mg) | Cholesterol (mg) | Entrapment Efficiency (%) | Particle size (nm) |
|-----------|--------------|------------|------------------|---------------------------|--------------------|
| B3        | 10           | 10         | 1                | 83                        | 182                |
| B7        | 30           | 20         | 3                | 58                        | 195                |
| B10       | 40           | 30         | 3                | 72                        | 161                |
| B11       | 60           | 50         | 5                | 81                        | 154                |
| B15       | 70           | 80         | 6                | 73                        | 178                |
| B17       | 90           | 100        | 8                | 77                        | 207                |
| B21       | 100          | 110        | 8                | 90                        | 101                |

## 5.10 FTIR study

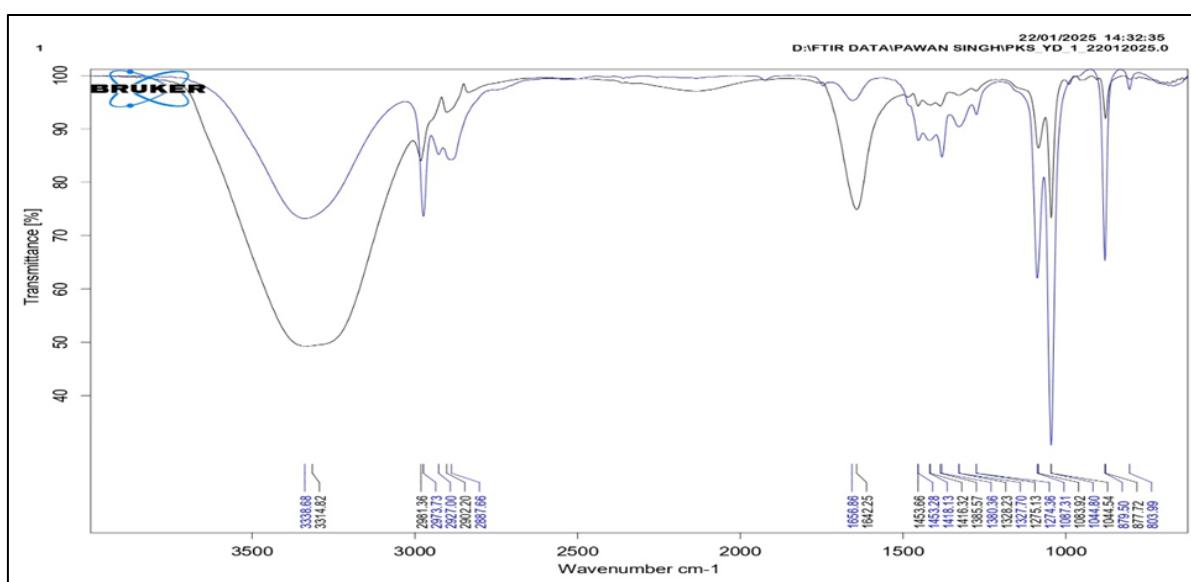
- FTIR analysis was performed to identify the functional groups present in the *Staphysagria* extract and confirm its chemical composition.
- It is the first step in rational formulation development. FTIR spectra of drug, excipients, and physical mixture of drug excipients were obtained.



Delphinine



| Functional Group | Frequency range (cm <sup>-1</sup> ) | Vibration type |
|------------------|-------------------------------------|----------------|
| O-H              | ~3300                               | stretching     |
| C-H              | ~2800–3000                          | stretching     |
| C=O              | ~1650–1750                          | stretching     |
| C=C              | ~1500–1600                          | stretching     |
| C-O              | ~1000–1300                          | stretching     |



## **Interpretation of FT-IR Spectra:**

- **Structural Integrity:** The primary functional groups of *Staphysagria* are retained in the formulation, confirming that the drug's core structure remains intact.
- **Drug-Excipient Interaction:** Reduced peak intensities and slight shifts suggest possible interactions (e.g., hydrogen bonding or dispersion effects) between the drug and excipients in the formulation.
- **Formulation Stability:** The similarity in spectral patterns between the drug and formulation suggests that the formulation process did not significantly alter the chemical properties of *Staphysagria*.

## **5.11 Evaluation of Phytosome**

### **a) Entrapment efficiency (%)**

- Entrapment efficiency (%) was determined to evaluate the proportion of *Staphysagriabioactives* successfully encapsulated within the phytosome formulation.
- This parameter is crucial for assessing the effectiveness of the phytosome-based drug delivery system in retaining and delivering the active ingredients for optimal therapeutic effects in the final shampoo formulation.

### **b) Particle size (nm)**

- The particle size of the formulated phytosome was determined to ensure uniformity, optimal entrapment efficiency, and effective delivery of the active constituents.
- The particle size analysis provided critical insights into the stability and performance of the phytosome in the shampoo formulation.

### **c) Zeta potential (mV)**

- Zeta potential analysis was conducted to evaluate the surface charge of the phytosome formulation, providing insights into its stability.

- A high zeta potential value indicates strong electrostatic repulsion between particles, which helps prevent aggregation and ensures the stability of the phytosome in the formulation.

#### **d) Release study of phytosome**

- A release study of the phytosome formulation was conducted using a dialysis membrane to assess the sustained release of *Staphysagria* bioactives.
- The study involved placing the phytosome formulation inside the dialysis membrane and monitoring the release of active ingredients over time.
- This method helps evaluate the controlled release profile and the potential of the phytosome formulation to provide prolonged therapeutic effects in the shampoo.



## **5.12 Formulation of Phytosomal shampoo**

### **a) Steps for Water-Based Hibiscus Extract**

- Fresh hibiscus flowers were rinsed thoroughly to remove dirt.
- Calyx and green parts were removed, keeping only the petals.
- Boil 1 liter of distilled water.
- 50 gms of fresh hibiscus petals is added to the boiling water.
- Reduce heat and let it simmer for 15–20 minutes.
- Allow the mixture to cool completely.
- Strain the liquid through a fine strainer to remove flower residue.

- The resulting liquid will be a deep red extract.

### b) Steps for preparation of phytosomal shampoo

- Coconut oil and castor oil were saponified with potassium hydroxide using a reflux condenser.
- After complete saponification, glycerine was incorporated with stirring followed by mixing of freeze dried phytosome.
- Ethyl alcohol, methyl paraben used as preservative and lemon grass oil used for masking the pungent smell of extract.
- Hibiscus extract as a colorant.

| Sr. No. | Ingredients            | Quantity (% w/w) |
|---------|------------------------|------------------|
| 1       | Castor oil             | 3                |
| 2       | Coconut oil            | 10               |
| 3       | Potassium hydroxide    | 3                |
| 4       | Ethyl alcohol          | 4                |
| 5       | Glycerin               | 2                |
| 6       | Staphysagria phytosome | 0.2              |
| 7       | Lemongrass oil         | 0.05             |
| 8       | Methyl paraben         | 0.01             |
| 9       | Distilled water q.s.   | 100              |
| 10      | Hibiscus extract       | 0.01             |

## 5.13 Characterization of prepared shampoo

### a) pH:

- 0.2% shampoo solution was used to determine the pH by using the pH meter.

### b) Foam Formation (Shake Test):

- 2 ml of 0.2% shampoo was mixed with water in a measuring cylinder. Then covered the cylinder with hand and shaken 10 times. Foam was retained for more than 15 minutes.

**c) Skin irritation test:**

- Applied the solution of prepared shampoo on skin and kept for 5 min and observed for redness of skin and irritation. There were no any red coloration and the irritation to the skin.

#### **5.14 Antimicrobial study of the prepared shampoo**

- The antimicrobial study of the prepared shampoo formulation was conducted using the agar well diffusion method (bore method).
- This method involved creating wells in an agar plate inoculated with microorganisms, followed by the addition of standard drug and the shampoo formulation to assess its ability to inhibit microbial growth.
- The zone of inhibition was measured to evaluate the antifungal and antibacterial efficacy of the shampoo against specific bacteria and fungi, providing insights into its potential for addressing scalp and hair-related microbial issues.

## **6. Results and Discussion**

### **1. Extraction of crude drug and calculating percentage yield:**

Extractive value of *Staphysagria* seed was found to be 17.84%.

### **2. Solubility analysis of extract:**

Extract obtained from *Staphysagria* seed was soluble in ethanol and insoluble in water.

### **3. Preliminary Phytochemical Screening of seed extract:**

- Preliminary phytochemical screening was conducted to identify the presence of bioactive constituents in *Staphysagria*.
- The analysis revealed the presence of key phytochemical which are alkaloids, which are known to contribute to its therapeutic properties.

### **4. Thin Layer Chromatography (TLC)**

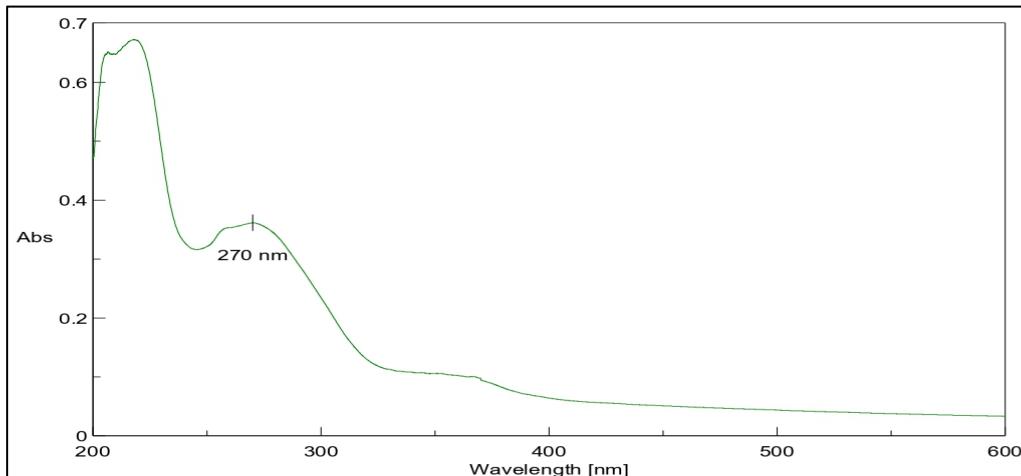
Rf value was found to be 0.55 which complies with Homoeopathic Pharmacopoeia of India Volume VI.

### **5. Determination of Total Alkaloidal Content**

Total alkaloid content was found to be 2.916 % w/w

### **6. Determination of maximum wavelength ( $\lambda_{\text{max}}$ ) and standardization of extract:**

The  $\lambda_{\text{max}}$  of *Staphysagria* extract was found to be 270 nm which complies with the  $\lambda_{\text{max}}$  of Delphinine as per Homoeopathic Pharmacopoeia of India Volume VI.



$\lambda_{\text{max}}$  of Staphysagria extract (1000 ppm)

## 7. Preparation of Phytosome:

Phytosome was prepared by solvent injection technique.

## 8. FTIR study:

### Interpretation:

- **Structural Integrity:** The primary functional groups of *Staphysagria* are retained in the formulation, confirming that the drug's core structure remains intact.
- **Drug-Excipient Interaction:** Reduced peak intensities and slight shifts suggest possible interactions (e.g., hydrogen bonding or dispersion effects) between the drug and excipients in the formulation.
- **Formulation Stability:** The similarity in spectral patterns between the drug and formulation suggests that the formulation process did not significantly alter the chemical properties of *Staphysagria*.

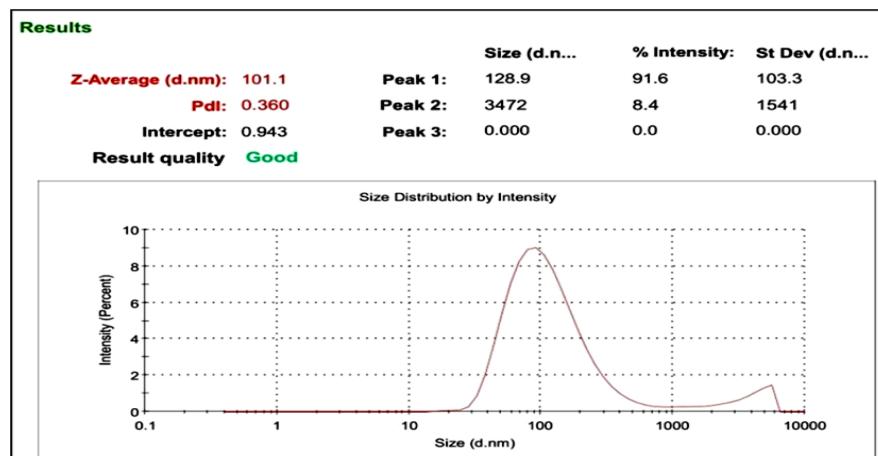
## 9. Evaluation of Phytosome:

### 9.1. Entrapment efficiency (%)

Entrapment efficiency of prepared phytosome was found to be 89.7% by Ultraviolet-visible (UV-Vis) spectroscopy.

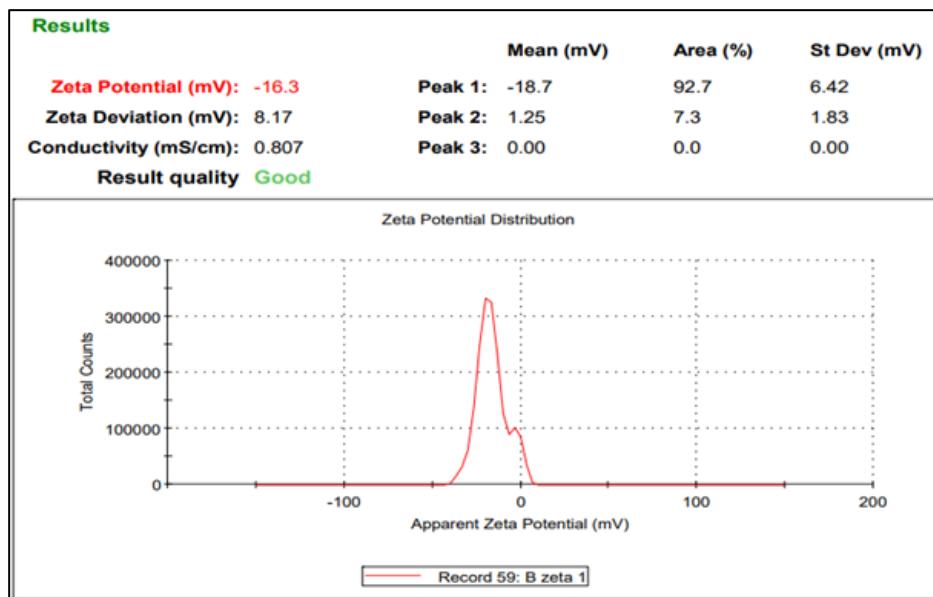
## 9.2. Particle size (nm)

Particle size of prepared phytosome was found to be 101.1 nm.



## 9.3. Zeta potential (mV)

Zeta potential of prepared phytosome was found to be -16.30 mV..

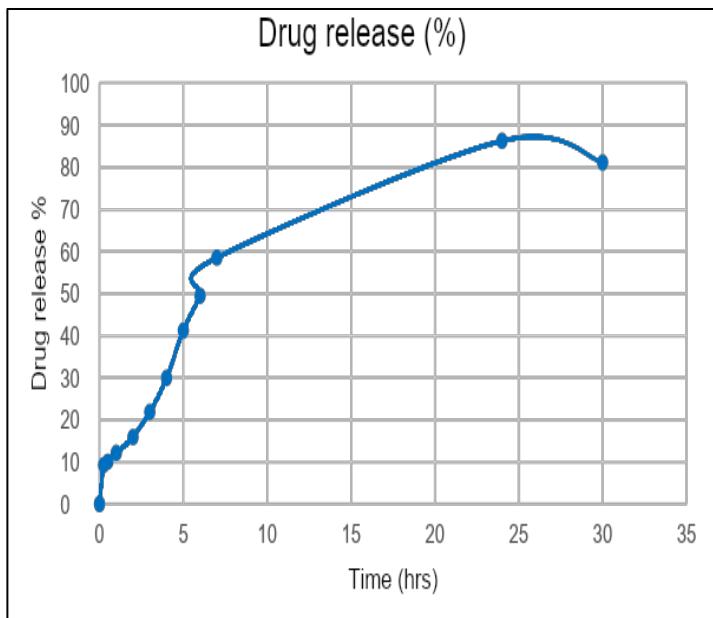


#### **9.4. Release study of phytosome**

Release study of prepared phytosome was done by Ultraviolet-visible (UV-Vis) spectroscopy.

Drug release (%) was found to be 86.31% at 24 hours.

| Time (hrs) | Drug release (%) |
|------------|------------------|
| 0          | 0                |
| 0.25       | 9.25             |
| 0.5        | 10.05            |
| 1          | 12.1             |
| 2          | 15.9             |
| 3          | 21.85            |
| 4          | 29.95            |
| 5          | 41.2             |
| 6          | 49.47            |
| 7          | 58.53            |
| 24         | 86.31            |
| 30         | 81.14            |



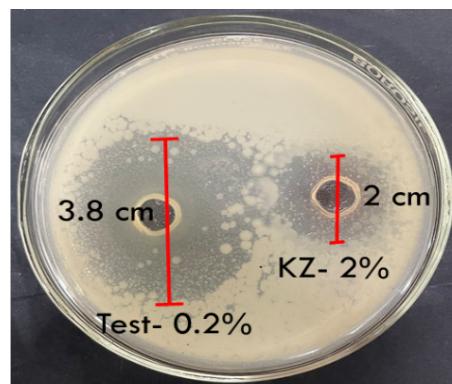
#### **10. Antimicrobial study of the prepared shampoo**

##### **10.1. Antifungal Activity**

The antifungal activity of the formulated *Staphysagria*-based shampoo (0.2%) was evaluated against *Candida albicans*, with ketoconazole (2%) used as the standard control. The agar well diffusion method was employed to assess antifungal efficacy by measuring the zone of inhibition.

The *Staphysagria*-based shampoo exhibited a significantly larger zone of inhibition in comparison to ketoconazole. This indicates a higher level of antifungal activity against *Candida albicans*.

The measured zone of inhibition for the *Staphysagria*-based shampoo was observed to be greater than that of the ketoconazole control, confirming its potential antifungal effectiveness.



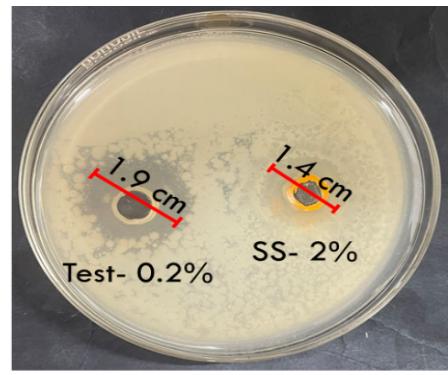
KZ- 2% = Ketoconazole 2% solution  
Test- 0.2% = *Staphysagria* shampoo 0.2%

## 10.2 Antibacterial Activity

The antibacterial activity of the formulated *Staphysagria*-based shampoo (0.2%) was evaluated against *Staphylococcus aureus*, with selenium sulfide (2%) used as the standard reference. The assessment was carried out using the agar well diffusion method, and the zone of inhibition was measured to determine antibacterial efficacy.

The *Staphysagria*-based shampoo exhibited a larger zone of inhibition compared to the selenium sulfide control, indicating a higher level of antibacterial activity against *Staphylococcus aureus*.

These results suggest that the *Staphysagria*-based shampoo demonstrates notable antibacterial potential against *S. aureus*, supporting its effectiveness in promoting scalp hygiene and preventing bacterial infections.



SS- 2% = Selenium Sulfide 2% solution  
Test- 0.2% = *Staphysagria* shampoo 0.2%

## 11. Formulation of *Staphysagria* enriched phytosomal shampoo

*Staphysagria* enriched phytosomal shampoo was prepared and evaluated for pH, foam stability and skin irritation test.

## 12. Evaluation of *Staphysagria* enriched phytosomal shampoo

### 12.1. pH:

The pH of the prepared shampoo was 6.4.

### 12.2. Foam stability test:

Foam was found stable for more than 15 minutes.

### 12.3. Skin irritation test

There was no red coloration and irritation to the skin.

## 7. LABEL

### Suggested use

Wet Hair and Scalp thoroughly  
Apply a generous amount of Staphysom  
Gentle Massage into scalp and leave for 3-5 minutes  
Rinse well with water  
Use 2-3 times per week or As Directed By Physician

### INDICATION

ANTIDANDRUFF & ANTIBACTERIAL

For external use only

Manufactured at  
Principal K M.Kundnani  
College of pharmacy  
Add-plot no -23, jote joy  
building, cuffe parade,  
Mumbai-400005



### Ingredients

|                        |        |
|------------------------|--------|
| Staphysagria Phytosome | 2.45%  |
| Castor oil             | 8.18%  |
| Coconut oil            | 2.45%  |
| Potassium hydroxide    | 3.27%  |
| Ethyl alcohol          | 1.64%  |
| Glycerin               | 0.16%  |
| Lemon grass oil        | 0.04%  |
| Methyl paraben         | 0.008% |
| Distilled water        | 81.79% |
| Hibiscus extract       | 0.008% |



BATCH NO- 21  
MFG DATE-JAN 2025  
EXP DATE-JAN 2027



## **8. Conclusion**

The *Staphysagria*-enriched NDDS shampoo offers a natural and effective solution for hair and scalp care. FTIR analysis and phytochemical screening confirmed the retention of *Staphysagria*'s bioactive properties. The phytosome demonstrated high entrapment efficiency (89.7%) and optimal particle size (101.1 nm). Sustained drug release was achieved, with 86.31% released over 24 hours. Superior antifungal activity against *Candida albicans* and antibacterial efficacy against *Staphylococcus aureus* were observed. The formulation outperformed conventional treatments like Ketoconazole and Selenium sulfide. This research sets a benchmark for eco-friendly and innovative herbal hair care products.

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