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# FORMULATION AND EVALUATION OF ANTI-DANDRUFF HERBAL SHAMPOO CONTAINING *DATURA METEL* [LINN] LOADED SOLID LIPID NANOPARTICLES

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## ABSTRACT

The aim of this present work was to develop anti-dandruff herbal shampoo containing *Datura metel* Linn loaded solid lipid nanoparticles. Dandruff is a chronic scalp condition characterized by scaling, itching, and redness of the scalp. Yeast like fungus *Malassezia furfur* was responsible for dandruff formation. *Datura metel* is a well-known medicinal plant due to its herbicidal, insecticidal, anti-fungal, anti-bacterial, anti-inflammatory and anti-rheumatoid activity. The leaves of *Hibiscus rosa-sinesis* also added into this herbal shampoo due to its greater conditioning effect on hair. The *Datura metel* loaded Solid Lipid Nanoparticles (SLNs) were prepared by high shear hot homogenization followed by ultrasonication method. The organoleptic evaluations of the formulations showed good results. The pH of the shampoo ranged between 5.47 to 6.10, which was near to the skin pH. The percentage solid content of prepared shampoos was found between 22.15-25.30 %. All the shampoos produced stable foams in distilled water with good conditioning performance which was due to the presence of *Hibiscus rosa-sinesis* leaf extracts. Viscosity and surface tension of developed formulations was found to be 7782.67-8867.87 cps and 30.54-33.16 dynes/cm respectively. All the developed formulations showed anti-fungal activity against *Malassezia furfur* but F3 produced a better zone of inhibition (32 mm) which was near to the zone of inhibition produced by standard Ketoconazole shampoo (36 mm). The optimized formulation F3 was found to be stable during the period of stability studies. Hence it was concluded that the formulated anti-dandruff herbal shampoo was safe and effective for controlling dandruff with a better conditioning effect.

**KEYWORDS**: Dandruff; Malassezia furfur; Datura metel Linn; Hibiscus rosa-sinesis Linn; Solid Lipid Nanoparticles (SLNs); Herbal anti-dandruff shampoo.

## INTRODUCTION

The word dandruff is of Anglo-Saxon origin, a combination of "tan" meaning "tetter" and "drof" meaning "dirty". Dandruff is a chronic scalp condition, which involves the excessive shedding of dead skin cells from the scalp. The skin of scalp renews itself about once in a month. Usually, scalp sheds dead cells in a nearly invisible way, but sometimes cell turnover becomes unusually rapid and dead cells are shed as visible flakes called dandruff. In the physiological spectrum of scaling, about 800,000 cell/cm$^2$ are released during dandruff and the number of cells reduced to 487,000 cells/cm$^2$ after detergent treatment. Dandruff can be characterized by scaling (presence of fragments), itching and redness around the scalp.[1,2]

**According to the symptoms, dandruff is classified into two types.[3,4]**

**1. Dry dandruff:** Also known as *Pityriasis simplex*. It is characterized by excessive formation white grayish or ashen colored minute scales. These scales are at first found in the middle of scalp area and then spread towards parietal, frontal and occipital areas. There is no excessive hair loss is observed in this condition.

**2. Oily dandruff:** Also known as *Pityriasis steatoides*. It arises on the scalp skin with varied intensity of sebum production. It appears as dirty yellow colored oily scales that can form lesions and hair fall is common in this condition. The most common site affected by this type of dandruff is scalp, between eyebrows, side of the nose, behind the ears, over the breastbone and sometimes in the armpits.

**There are three factors involved in the formation of dandruff[5-8]**

**1. Sebaceous gland secretions**

Dandruff occurs exclusively on the skin in areas with high levels of sebum. Human sebum is a complex mixture of cholesterol, cholesterols esters, sterol esters, wax esters, triglycerides, fatty acids, and squalene. When sebum is secreted, triglycerides and esters are converted into monoglycerides, diglycerides, and free fatty acids by microbes. The free fatty acids induce an irritant response, which is involved in scalp hyperproliferation. Stress and hormones also play a key role in sebum secretion, which in turn increases the severity of dandruff formation.

**2. Malassezia fungi**

The lipophilic yeast belonging to the genus *Malassezia* is responsible for dandruff formation [Figure:3]. The name *Malassezia* is originated by Malassez in 1898, during the 20$^{th}$ century this genus was renamed to *Pityrosporum*. There are seven species of *Malassezia* which have been recognized in dandruff formation. They are *M. globosa*, *M. resticta*, *M. obtuse*, *M. sloofiae*, *M. sympodialis*, *M. furfur* and *M. pachydermatis.* The levels of *Malassezia* increased by 1.5 to 2 than its normal level during dandruff.

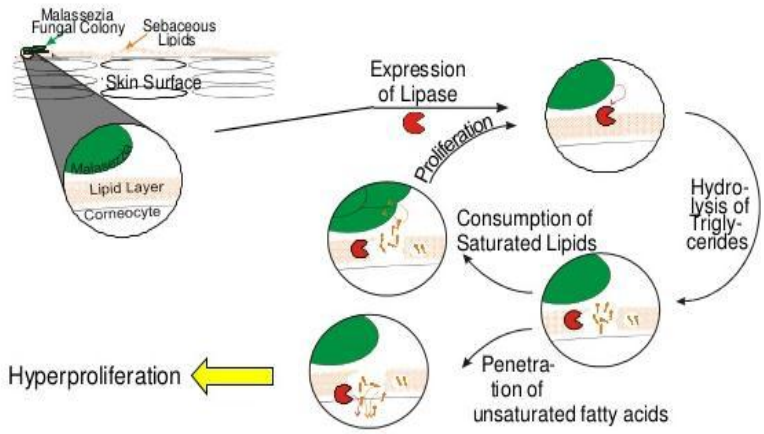


**Figure. 1: The role of *Malassezia* lipid metabolism in dandruff genesis.**

**3. Individual susceptibility:** It mainly depends on how the individuals are genetically susceptible to the dandruff condition.

Dandruff can be controlled and effectively treated by using various formulations. The formulation must be suitable for the hairy region and it should be compatible with the dandruff condition. There are different types of formulations like shampoos, creams, lotions, emulsions, hair oils and other cosmetic formulations containing anti-dandruff agents are readily available in the market to control dandruff. Out of which shampoos are the most common as well as a worldwide accepted cosmetic product used against dandruff. A shampoo can be defined as "a preparation of a surfactant (i.e. surface active material) in suitable forms like liquid, solid or powder, which when used under the conditions specified will remove surface grease, dirt, and skin debris from the hair shaft and scalp without affecting adversely the hair, scalp, or health of the user". It also imparts other benefits such as lubrication, hair conditioning, medication etc. One of the main functions of shampoo is to remove dandruff from the hair and it is achieved by detergents.

The anti-dandruff shampoo is a type of shampoo which contains anti-dandruff agent and it is mainly used to prevent or treat dandruff from the scalp of hair. Herbal anti-dandruff shampoos are the cosmetic formulations which contain herbal ingredients such as plant extracts and essential oil. These herbal shampoos are generally used to remove the dandruff, to add natural colour to the hair, to remove the extra oil content of the hair, for the healthy growth of the hair, to remove the dust, dirt and scales of the scalp, to prevent hair falling, to impart softness and smoothness to the hair shaft etc. It is assumed that they can penetrate to the root shafts, stimulate the sebaceous glands and enhance the blood circulation and impart greater strength to the hair root and the shaft. They are also used against alopecia, thinning, clubbing and greying of hair and hair shaft roughness and breaking.[9]

## MATERIALS AND METHODS

### Collection of plants

The fresh leaves of *Datura metel* and *Hibiscus rosa-sinesis* were collected from Kasaragod district, Kerala (India) in the month of October 2016. The plant materials were identified and authenticated by Dr. A Rajagopalan, Professor, Department of Horticulture, College of Agriculture, Padannakad, Kasaragod, Kerala.

### Preparation of plant extracts[10,11]

The collected plant leaves were carefully washed under running tap water followed by sterilized distilled water and was air dried at room temperature for 30-45 days. These shade dried plant materials were then homogenized to a fine coarse powder using an electronic blender and then stored in air tight containers until further use. Various organic solvents viz. petroleum ether, chloroform, ethanol and water were used for extraction. 10gm of homogenized coarse leaf powders of *Datura metel* and *Hibiscus rosa-sinesis* were soaked in different conical flasks containing 100mL of petroleum ether, chloroform, ethanol and water. Then it is allowed to stand for 30 min in a water bath with occasional shaking, finally, each sample extract (petroleum ether, chloroform, ethanol and water) was filtered through sterilized Whatman No:1 filter paper and concentrated to dryness. Thus the obtained dried extracts were stored at $4^{o}$C in labeled sterile bottles until further use. To detect various biologically active constituents, present in various solvent extracts the standard methods were followed.

**Preliminary Phytochemical Screening**[12]

Phytochemical examinations for the leaf extracts of *Datura metel* and *Hibiscus rosa-sinesis* were carried out as per the standard methods.

**Extraction of Plant Materials**[10,11]

The collected plant leaves of *Datura metel and Hibiscus rosa-sinesis* were shade dried at room temperature and coarsely powdered. Both drugs were individually extracted by continuous hot extraction (soxhlation) using 95% ethanol in soxhlet apparatus. The process lasts for 2-4 days until the solvent present in siphon tube becomes colourless. Ethanol retained within the extract can be recovered by distillation process and it was then air dried and concentrated. This concentrated extracts of the two different drugs were then incorporated into different formulations.

**Preformulation Study**[13,14]

The FT-IR spectrum of drug extracts with other ingredients was analyzed for compatibility study.

**Formulation of anti-dandruff shampoo containing *Datura metel* loaded SLNs**

**Preparation of *Datura metel* loaded SLNs**[14-16]

SLNs were prepared by using high shear hot homogenization followed by ultrasonication method. The formula was given in Table no 1.

**Table no. 1: Composition of developed SLNs.**

| Formulation Code | Drug [g] | Lipid [g] | Surfactant [%] | Water [mL] |
|---|---|---|---|---|
| SLN 1 | 2 | 2 | 1.5 | Q.S |
| SLN 2 | 4 | 4 | 1.5 | Q.S |
| SLN 3 | 6 | 6 | 1.5 | Q.S |

Stearic acid was melted to at least $10^0$C above its melting point, ie; $75^0$C (melting point of Stearic acid = 69-$70^0$C)**.** The drug (*Datura metel*) was added to this melt of lipid and dissolved by stirring until the melt appeared clear. An aqueous phase was prepared by dissolving surfactant (Tween 80) in distilled water (sufficient to produce 100 mL) and heated to the same temperature of lipid phase ($75^0$C). Hot aqueous phase was added into lipid phase. Homogenization is carried out at 2100 rpm for 15 minutes by using mechanical stirrer and the temperature is maintained at $75^0$C. Hot oil in water emulsion was formed, which is subjected for ultrasonication for 30 minutes. Finally, this nanoemulsion is cooled to room temperature

to obtain *Datura metel* loaded SLNs. Then it is stored in the refrigerator in cool condition as the shelf life of SLNs dispersion is more at the cool condition as compared to room temperature.

**Preparation of anti-dandruff herbal shampoo[17]**

The anti-dandruff herbal shampoo was formulated by using simple mixing process. The shampoo was prepared by adding required amount of ingredients given in Table no 2.

**Table no. 2: Composition of developed anti-dandruff herbal shampoo.**

| Sl No | Ingredients | F1 | F2 | F3 |
|---|---|---|---|---|
| 1 | DM-SLNs | **SLN 1** | **SLN 2** | **SLN 3** |
| 2 | HR leaf extract | 2 % | 2 % | 2 % |
| 3 | CMC | 2 % | 2 % | 2 % |
| 4 | SLS | 15% | 15% | 15% |
| 5 | Glycerin | 5% | 5% | 5% |
| 6 | Methyl paraben | 0.25 % | 0.25 % | 0.25 % |
| 7 | EDTA | 0.5 % | 0.5 % | 0.5% |
| 8 | Lemon oil | 5 % | 5 % | 5 % |
| 9 | Rose oil | Q.S | Q.S | Q.S |
| 10 | NaCl | Q.S | Q.S | Q.S |
| 11 | Distilled water | Q.S | Q.S | Q.S |

*Datura metel* loaded SLNs and *Hibiscus rosa-sinesis* leaf extract was mixed with ingredients like carboxy methyl cellulose [2% gel], lemon oil, sodium lauryl sulfate, glycerin and a solution is made in a clean stainless steel or non-leaching glass container. Methyl paraben and EDTA were separately dissolved in a little amount of water and added into SLNs containing the solution. The viscosity of the shampoo is adjusted by adding a saturated solution of NaCl drop wise into it. Finally, perfume is added and the shampoo is packed in a suitable container for evaluation.

**Evaluations**

**4.10.1 Evaluation of *Datura metel* loaded SLNs[13, 14,17]**

**A. SEM:** The shape and morphological characters were obtained from SEM photographs of the optimized SLNs. The formulations were placed into circular aluminum stubs using double adhesive carbon tape and coated with gold in HITACHI ION SPUTTER E-1010 Vacuum evaporator. Then it was observed in HITACHI SU6600 FE SEM (field emission scanning electron microscope) having acceleration voltage of 10.0kV and magnification of 60.0k-100.0k.

### 4.10.2 Evaluation of anti-dandruff herbal shampoo[18-21]

Evaluation is important to determine the quality of formulated herbal anti-dandruff shampoo.

**There are mainly five different types of evaluations have been done, namely**

A. Organoleptic evaluations
B. Physico-chemical evaluations
C. Rheological property evaluations
D. *In-vitro* anti-dandruff activity evaluations
E. Stability studies.

**A. Organoleptic Evaluations**

The developed herbal anti-dandruff shampoos were evaluated for their organoleptic characters like colour, odor, texture and physical appearance.

**B. Physico-Chemical Evaluations**

**1. pH:** The formulated anti-dandruff shampoos were diluted by using distilled water to produce a shampoo of 10% v/v concentration. The pH of the prepared shampoos was determined by using digital pH meter at room temperature 30 ± $2^0$C.

**2. Percentage solid content:** A China dish was cleaned, dried and weighed. To this, an accurately weighed amount of formulated shampoo (4g) was added and the dish with shampoo was weighed. The china dish with shampoo was placed on a hot plate and heated until the liquid portion was evaporated. The weight of the solid contents of shampoo after complete drying was calculated.

**3. Cleaning action:** 5 grams of wool yarn was placed in grease and it was added into a flask containing 200 mL of water with 1 gram of shampoo. The temperature of the flask was maintained at 30 ± 2°C. Then the flask was shaken for 4 minutes at the rate of 50 shakes per minute. The solution was removed and the sample was taken out, dried and weighed. The amount of grease removed was calculated by using the following equation.

**DP= 100(1-T/C)**

Where,

DP = percentage of detergency power

C = weight of sebum in the control sample

T = weight of sebum in the test sample

**4. Wetting action**

Canvas disc sinking test is used to determine the wetting action of shampoo. Here the canvas was cut into 1-inch diameter discs with an average weight of 0.44 gram. The disc was placed on the surface of shampoo solution (1% v/v) and the stopwatch started. The time required for the disc to begin to sink was measured accurately and noted as wetting time.

**5. Foaming ability and foam stability**

Cylinder shake method was used to determine the foaming ability. 50mL of the 1% v/v shampoo solution was put into a 250 mL graduated measuring cylinder and it is covered with a hand. Measuring cylinder was shaken for 1 minute and the total volume of the foam contents after 1 minute of shaking was recorded. Foam stability was evaluated by recording the foam volume immediately after the shake test at 1 min intervals for 4 min.

**6. Dirt dispersion**

Two drops of shampoo were added to a large test tube containing 10mL of distilled water. To this solution, one drop of India ink was added and the test tube was stoppered and shaken 10 times. The amount of ink in the foam was indicated as None, Light, Moderate or Heavy.

**7. Conditioning performance**

A hair tress of a woman was obtained from a local saloon. It was cut into four swatches of the tresses with approximately the length of 10 cm and the weight of 5 g. A swatch without washing served as the control. Other three tresses were washed with the formulated shampoos. For each cycle, each tress was shaken with the mixture of 20 mL of sample and 20 mL of water in a conical flask for 5 min and then rinsed with 50 mL water. Afterward, each tress was left for air drying at room temperature. The tresses were washed for maximum ten cycles. The 10 student volunteers were invited to be the referees for determining the conditioning performance of the shampoos i.e. smoothness and softness of the tresses by a blind touch test. All the students were blind folded and asked to touch and rate the four tresses for conditioning performance. For scoring, they were asked to mark the conditioning performance of the tresses after contacting in 4-choice of satisfaction levels, i.e., excellent (score = 4), good (score = 3), fair (score = 2) and poor (score = 1). Identical scores were allowed.

**C. Rheological Property Evaluations**

**1. Viscosity:** The viscosity of the shampoos was determined by using Brookfield Viscometer (DV-1 programmable rheometer) at 10 rpm. The temperature and sample containers size was kept constant during the study.

**2. Surface tension:** Dilute the shampoo using distilled water to produce 10% v/v as concentration. Measurements were carried out at room temperature using stalagmometer. The stalagmometer was cleaned by using chromic acid and purified water. Dip the flattened end of stalagmometer into a beaker containing a sample of developed shampoo and suck it until the level reaches the mark. Fix the device in the stand and allow the sample to run slowly from the mark. Count the number of drops formed when the level of liquid reaches from A to B. Repeat the experiment with distilled water. The surface tension of shampoo was calculated using following equation.

$$R_2 = \frac{(W_3 - W_1)N_1}{(W_2 - W_1)\,N_2} \times R_1$$

Where,

$W_1$ = Weight of empty beaker.

$W_2$ = Weight of beaker with distilled water

$W_3$ = Weight of beaker with shampoo solution.

$N_1$ = No. of drops of distilled water.

$N_2$ = No. of drops of shampoo solution.

$R_1$ = Surface tension of distilled water at room temperature.

$R_2$ = Surface tension of shampoo solution

**D. *In-Vitro* Anti-Dandruff Activity Evaluations**

❖ **Agar well diffusion method**

Anti-dandruff activity of the developed shampoo was determined by agar plate well diffusion method. This method involves.

- **Preparation of pre-inoculum**

Take the loopful culture of *Malassezia furfur* aseptically and transfer to sterilized and cooled 25mL Sabouraud dextrose (broth) and mix well. Incubate the broth at $25^0$C for 24 hrs.

- **Preparation of pour plates**

A Sabouraud dextrose agar (150 mL) is autoclaved and poured to the already autoclaved plate and cooled to room temperature and allowed to solidify. The culture was spread on the agar surface aseptically by using sterilized cotton.

- **Making wells on agar plates**

Wells of 6 mm in diameter were made aseptically on the agar plate by using a sterilized well digger. The shampoo samples (100 $\mu$L) were aseptically added into the well by using a micropipette. The petri plates are kept in a refrigerator (1 hour) for the diffusion of substances from well into surrounding medium. During this time the growth of the organism is reduced. After 1 hour, the plates were incubated in inverted condition at $37^0$C for 48 hours.

- **Measurement of the zone of inhibition**

After 48 hours, the plates were observed for the presence of inhibition of fungal growth and it was indicated in the form of a clear zone of inhibition around each well containing different samples. The size of the inhibitory zone was measured in "mm". The zone of inhibition obtained for the developed herbal anti-dandruff shampoo was compared with the standard. Ketoconazole (2% w/v) shampoo was used as a standard.

**E. Stability Studies**

The optimized formulation was subjected to stability studies in accordance with ICH guidelines for accelerated testing with required modification. The sample from each formulation was taken and kept at room temperature ($25\text{-}30^0$C $\pm$ $2^0$C) as well as a refrigerator ($2\text{-}8^0$C $\pm$ $3^0$C) for the duration of three months. The samples were tested and repacked for their physical appearance, pH, viscosity, cleaning action, foam stability and antimicrobial studies at an interval of one month.

## RESULTS AND DISCUSSIONS

### 1. Preparation of plant extracts

The collected plant materials were subjected to extraction using various solvents such as petroleum ether, chloroform, ethanol and water. The extracts obtained after maceration process was then used for phytochemical studies to choose the most suitable solvent for further extraction.

**2. Preliminary Phytochemical Screening**

Standard procedures were followed in order to determine the therapeutically active constituents present in the extracts and the results obtained were mentioned in Table no 3 & 4.

**Table no. 3: Phytochemical test on various extracts of *D. metel* leaf powder.**

| Phytochemical Test | Ethanolic Extract | Chloroform extract | Petroleum ether extract | Aqueous Extract |
|---|---|---|---|---|
| Alkaloids | +++ | ++ | + | - |
| Glycosides | + | + | - | - |
| Flavanoids | +++ | ++ | + | + |
| Carbohydrates | + | + | - | + |
| Saponins | ++ | + | - | + |
| Phytosterols | ++ | - | - | + |
| Tannins | +++ | + | + | ++ |
| Phenols | +++ | - | - | - |
| Proteins and amino acids | + | - | - | - |

**Table no. 4: Phytochemical test on various extracts of *H. rosa-sinesis* leaf powder.**

| Phytochemical Test | Ethanolic Extract | Chloroform extract | Petroleum ether extract | Aqueous Extract |
|---|---|---|---|---|
| Alkaloids | +++ | ++ | + | + |
| Glycosides | ++ | + | + | - |
| Flavanoids | +++ | + | - | + |
| Carbohydrates | +++ | - | - | - |
| Saponins | ++ | + | + | + |
| Phytosterols | +++ | - | - | + |
| Tannins | + | - | - | - |
| Phenols | + | + | - | + |
| Proteins and amino acids | + | - | - | - |

**(+++)** abundant, (++) moderate, (+) present, (-) absent

The phytochemical test on various extracts of *Datura metel and Hibiscus rosa-sinesis* leaf powder indicates the presence of various biologically active constituents and ethanolic extracts showed comparatively better results than other solvents. The present study confirmed that ethanol can be used as menstrum for further extraction.

**3. Extraction of plant materials**

The extraction of dried leaves of *Datura metel and Hibiscus rosa-sinesis* were carried out by continuous hot Soxhlet extraction process by using ethanol as solvent. The extracts obtained were collected and concentrated which was then weighed and kept in a desiccator until it was used for further studies. The yield so obtained was shown in Table no.5.

**Table no 5: Percentage yield of the extracts.**

| Sl. No | Plants | Solvent | Weight of dry powder (g) | Weight of dry extract (g) | Percentage Yield (% w/w) |
|---|---|---|---|---|---|
| 1 | *D.metel* | Ethanol | 50 | 14.30 | 28.60 |
| 2 | *H.rosa-sinesis* | Ethanol | 50 | 12.09 | 24.18 |

**(+++)** abundant, (++) moderate, (+) present, (-) absent.

**Table no 6: Physical characteristics of the extracts.**

| Sl. No | Extract | Colour | Odour | Consistency |
|---|---|---|---|---|
| 1 | *D.metel* | Dark green | Characteristic | Thick semisolid |
| 2 | *H.rosa-sinesis* | Dark green | Characteristic | Thick semisolid |

## 4. Preformulation study

❖ Drug- Excipient Compatibility Studies by FT-IR.

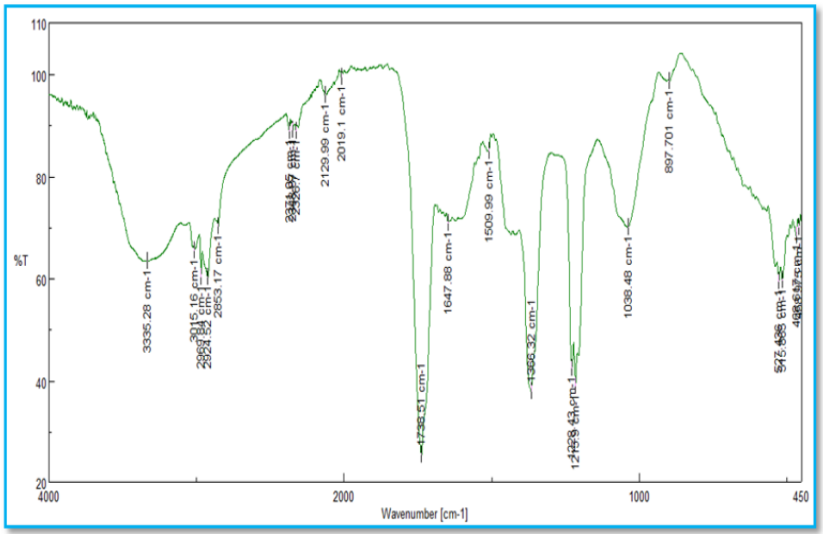


**Figure. 2: FT-IR Spectrum of *Datura metel* Linn.**

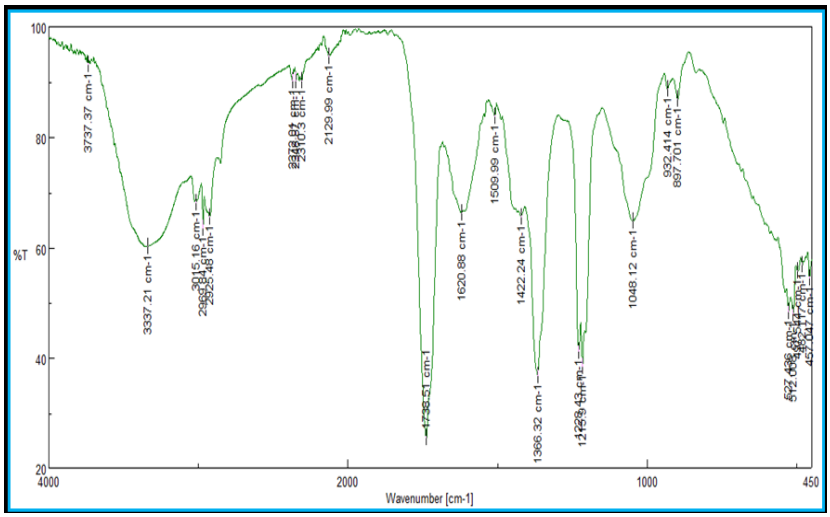


**Figure. 3: FT-IR Spectrum of *Hibiscus rosa-sinesis* Linn.**

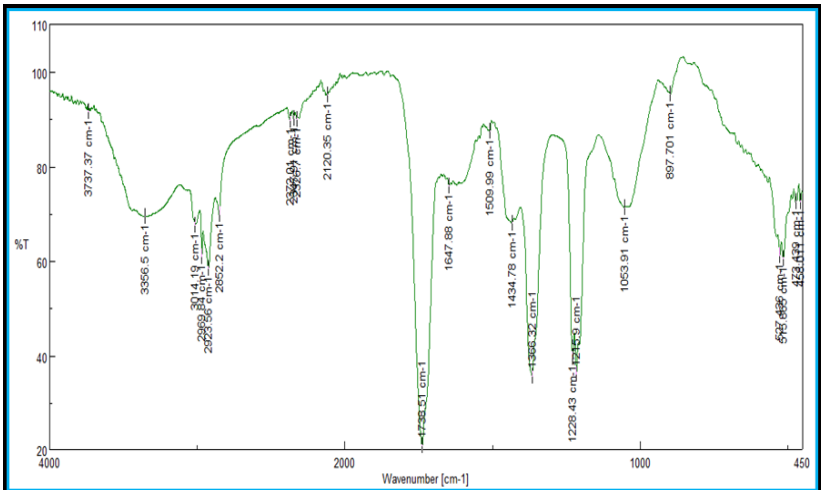


**Figure. 4: FT-IR Spectrum of developed formulation.**

**5. Formulation of anti-dandruff shampoo containing *Datura metel* loaded SLNs**

**5.1 Preparation of Datura metel loaded SLNs:** The SLNs were prepared by using high shear hot homogenization followed by ultrasonication method.

**6. Evaluation**

**6.1 Evaluation of *Datura metel* loaded SLNs:-**

**A. SEM**

Prepared SLNs were subjected for morphological studies using Field Emission Scanning Electron Microscope having an acceleration voltage of 10.0 kV and magnification of 100 k. SEM photographs are shown in Figure No:20.

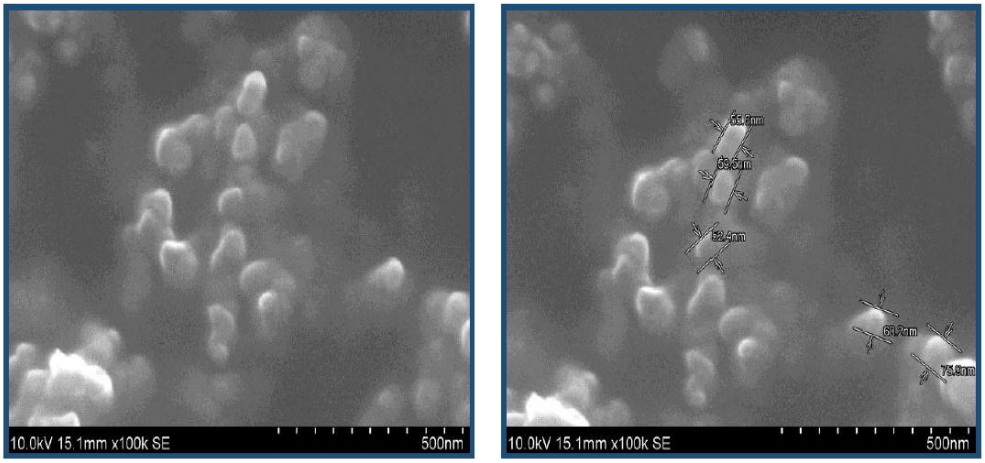


**Figure. 5: SEM photographs of formulated SLNs.**

**Evaluation of herbal anti-dandruff shampoo**

**A. Organoleptic Evaluations**

The organoleptic parameters like colour, odour, texture and physical appearance were evaluated by visual inspection and the results are shown in Table no 7.

**Table no. 7: Organoleptic evaluations of anti-dandruff herbal shampoo.**

| Formulation code | Colour | Odour | Texture | Physical Appearance |
|---|---|---|---|---|
| F1 | Light green | Characteristic | Smooth | Clear, thick liquid |
| F2 | Dark green | Characteristic | Smooth | Clear, thick liquid |
| F3 | Dark green | Characteristic | Smooth | Clear, thick liquid |

**B. Physico-Chemical Evaluations**

**1. pH:** The pH of 10% v/v shampoo solution in distilled water was determined at room temperature by using a digital pH meter and the values are given in Table no 8. The pH of all the developed formulations ranges from 5.47 to 6.10, which lies within the normal pH range of scalp (4.5-5.5). The pH of formulation F3 was found almost near to this pH level (5.47 ± 0.27).

**Table no 8: pH of developed anti-dandruff herbal shampoo.**

| Formulation code | pH |
|---|---|
| F1 | 5.80 ± 0.58 |
| F2 | 6.10 ± 0.18 |
| F3 | 5.47 ± 0.27 |

*All values are expressed as average ± SD; (n=3)

**2. Percentage solid content**

If the shampoo has too many solids, it will be difficult to work into the hair or too hard to wash out. The results of the percentage solid contents (Table no 9) was found between 22.15-25.30%. Out of these three developed formulations, F1 shows the minimum percentage solid content of 22.15%. It indicates that F1 may be a product which is easier to wash out.

**Table no 9: Percentage solid content of the formulation.**

| Formulation Code | Percentage solid content (%) |
|---|---|
| F1 | 22.15 ± 0.42 |
| F2 | 24.43 ± 0.79 |
| F3 | 25.30 ± 0.87 |

*All values are expressed as average ± SD; (n=3).

**3. Cleaning action:** Cleaning action was tested on wool yarn in grease. As seen from the result (Table no: 10), there is a significant difference in the amount of sebum removed by the different shampoos. F3 possess greater cleaning action (74.89 ± 0.65%) than other two formulations.

**Table no. 10: Cleaning action of formulation.**

| Formulation Code | Cleaning action (%) |
|---|---|
| F1 | 40.17 ± 0.77 |
| F2 | 45.45 ± 0.44 |
| F3 | 51.11 ± 0.38 |

*All values are expressed as average ± SD; (n=3)

**4. Wetting action:** The wetting action was measured by canvas disc sinking test. The time taken for sinking was given as wetting time. If the time required for sinking is less, then the wetting efficiency will be high. The result shows that formulation F3 requires less wetting time (171± 0.64), hence it has higher wetting efficiency.

**Table no 11: Wetting action of formulation.**

| Formulation Code | Wetting Time (Sec) |
|---|---|
| F1 | 175.25 ± 0.88 |
| F2 | 182.77 ± 0.54 |
| F3 | 171.66 ± 0.64 |

*All values are expressed as average ± SD; (n=3)

**5. Foaming ability and foam stability**

Foaming ability was determined by using cylinder shake method. All the three shampoos showed similar foaming properties in distilled water. The foam stability of herbal shampoos after 4 minutes is presented in Table no 12 and Figure:6. The results confirms that all the formulations produced stable foams with little bit change in foam volume.

**Table no. 12: Foam ability and foam stability of formulation.**

| Time (min) | Foam volume (mL) | | |
|---|---|---|---|
| | F1 | F2 | F3 |
| 1 | 159.7 ± 0.36 | 155.7 ± 0.66 | 150.6 ± 0.64 |
| 2 | 156.0 ± 0.15 | 154.0 ± 0.64 | 149.4 ± 0.6 |
| 3 | 153.1 ± 0.32 | 152.1 ± 0.98 | 147.8 ± 0.35 |
| 4 | 152.4 ± 0.65 | 149.2 ± 0.77 | 147.0 ± 0.45 |
| 5 | 149.1 ± 0.26 | 145.1 ± 0.7 | 144.3 ± 0.66 |

*All values are expressed as average ± SD; (n=3)

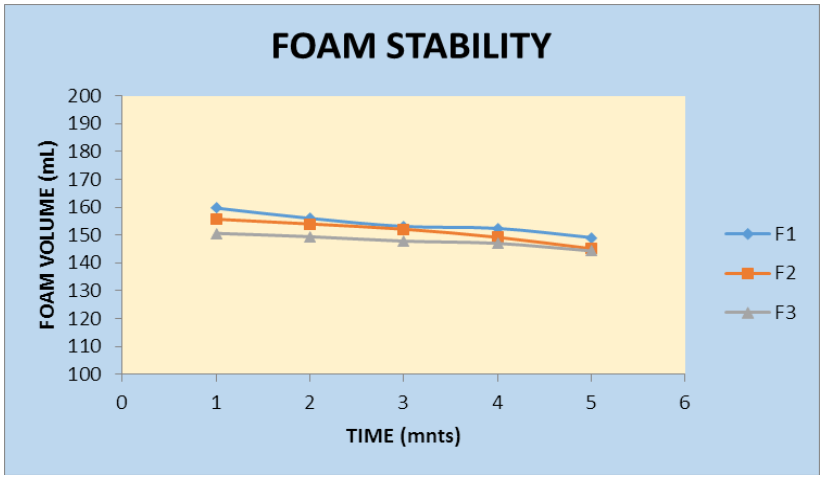


**Figure. 6: Graphical representation of foam stability.**

**6. Dirt dispersion:** Dirt dispersion is an important parameter for the evaluation of shampoo. Shampoo that causes the ink to concentrate in the foam was considered of poor quality. Because the ink or dirt that stays in foam is difficult to rinse away and gets redeposit on the hair. The results indicated that there is no dirt retained in the foam, so the developed shampoos were found to be satisfactory.

**Table no 13: Dirt dispersion of formulation.**

| Formulation code | Dirt dispersion |
|---|---|
| F1 | None |
| F2 | None |
| F3 | None |

**7. Conditioning performance:** Conditioning performance of developed shampoos was evaluated by blind touch test. The mean scores of students volunteer for conditioning performance is shown in Table no 14. The mean score of the conditioning performance of tress washed with formulated shampoo (F3) was found to be 3 and control tress (without washing) got the minimum score 1.1 out of 4.

**Table no 14: Conditioning performance of shampoo.**

| Score | F1 | F2 | F3 | Control |
|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 9 |
| 2 | 3 | 3 | 1 | 1 |
| 3 | 7 | 6 | 8 | 0 |
| 4 | 0 | 1 | 1 | 0 |
| Mean | 2.7 | 2.8 | 3 | 1.1 |
| Score 4 = Excellent; Score 3 = Good; Score 2 = Fair and Score 1 = Poor | | | | |

## RHEOLOGICAL PROPERTY EVALUATIONS

**1. Viscosity:** The viscosity of the prepared shampoo was measured by using Brookfield viscometer. The measurements were performed in triplicate at room temperature and the viscosity profile of the shampoo was recorded in Table no 15. Viscosity of the formulation was found in the range of 7782.67 to 8867.87 cps.

**Table no. 15: Viscosity of shampoo.**

| Formulation Code | Viscosity (cps) |
|---|---|
| F1 | 7782.67 ± 0.92 |
| F2 | 8587.15 ± 0.64 |
| F3 | 8867.87 ± 0.73 |

*All values are expressed as average ± SD; (n=3)

**2. Surface tension:** The surface tension of 10% solution of each sample in water was evaluated by using a stalagmometer. A shampoo is considered of good quality if it decreases the surface tension of pure water from 72.28 dynes/cm to about 40 dynes/cm. All tested shampoos showed a similar reduction in surface tension ranged from 30.54 to 33.16 dynes/cm.

**Table no 16: Surface tension of shampoo.**

| Formulation Code | Surface tension (dynes/cm) |
|---|---|
| F1 | 33.16 ± 0.70 |
| F2 | 32.95 ± 0.43 |
| F3 | 30.54 ± 0.55 |

*All values are expressed as average ± SD; (n=3)

## D. *IN-VITRO* ANTI-DANDRUFF ACTIVITY EVALUATIONS

The anti-dandruff activity of the developed formulation was done by using agar well diffusion method. The anti-fungal activity was measured in terms of zone of inhibition. The zone of inhibition obtained for various formulations was compared with the standard Ketoconazole shampoo (2% w/v). All the developed formulations showed anti-fungal activity against *Malassezia furfur* but F3 produced a better zone of inhibition of about 32 mm which was near to the zone of inhibition produced by standard Ketoconazole shampoo (36 mm).

**Table no. 17: Measurement of zone of inhibition.**

| Sl No | Test samples | Zone of inhibition (mm) |
|---|---|---|
| 1 | *Datura metel* extract (10mg/ml) | 8 mm |
| 2 | SLN dispersion of *Datura metel* | 12mm |
| 3 | Herbal anti-dandruff shampoo F1 | 18mm |
| 4 | Herbal anti-dandruff shampoo F2 | 24mm |
| 5 | Herbal anti-dandruff shampoo F3 | 32mm |
| 6 | Ketoconazole shampoo (standard) | 36mm |

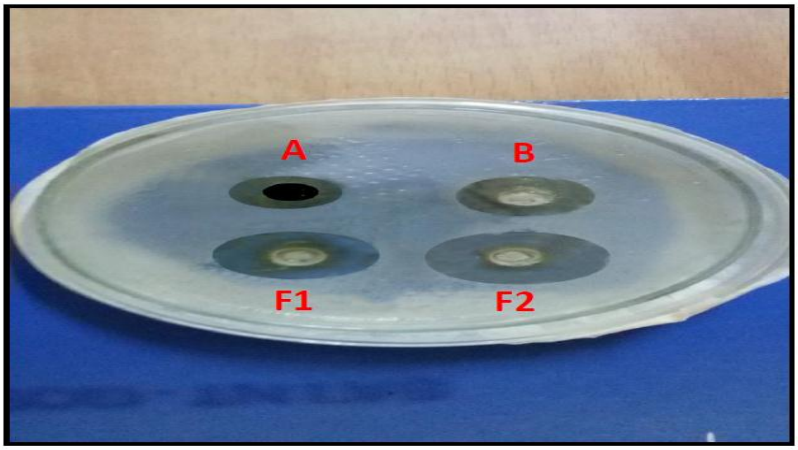


**Figure. 7: Zone of inhibition of *Datura metel* extract (A), SLN dispersion of *Datura metel* (B), herbal antidandruff shampoo (F1), and herbal anti-dandruff shampoo (F2).**

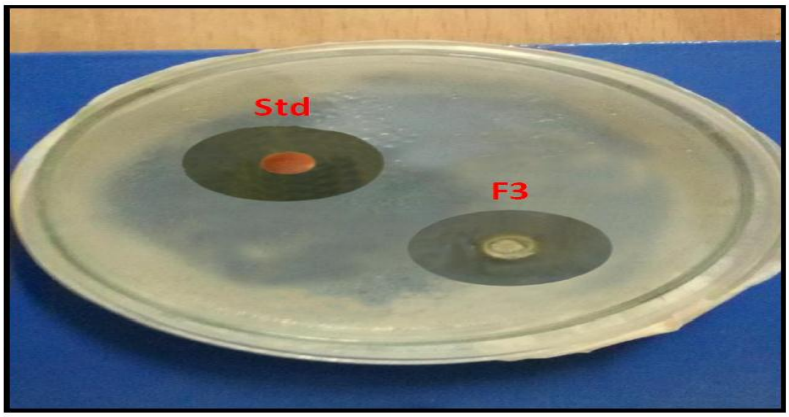


**Figure. 8: Zone of inhibition for herbal anti-dandruff shampoo (F3) and standard Ketoconazole shampoo (Std).**

**Stability Studies**

The optimized formulation (F3) was placed in a suitable borosilicate container and stored at room temperature 25-30°C ± 2°C and in the refrigerator at 2-8°C ± 3°C for a period of three months. The results obtained are shown in Table no 18.

**Table no. 18: Stability study of anti-dandruff shampoo (F3) at 25 - 30°C ± 2°C.**

| Sl. No | Evaluation Parameters | After one month Observation | After two month Observation | After three month Observation |
|---|---|---|---|---|
| 1 | Colour | Dark green | Dark green | Dark green |
| 2 | Appearance | Thick liquid | Thick liquid | Thick liquid |
| 3 | pH | 5.46 ± 0.39 | 5.45 ± 0.38 | 5.42 ± 0.39 |
| 4 | Foam volume (mL) | 150.13 ± 0.30 | 148.03 ± 0.70 | 149.56 ± 0.30 |
| 5 | Dirt dispersion | None | None | None |
| 6 | Viscosity (cps) | 8867.89 ± 0.95 | 8867.46 ± 0.44 | 8867.51 ± 0.69 |
| 7 | Surface tension (dynes/cm) | 30.83 ± 0.25 | 29.84 ± 0.34 | 30.15 ± 0.53 |

**Table no. 19: Stability study of anti-dandruff shampoo (F3) 2-8°C ± 3°C.**

| Sl. No | Evaluation Parameters | After one month Observation | After two month Observation | After three month Observation |
|---|---|---|---|---|
| 1 | Colour | Dark green | Dark green | Dark green |
| 2 | Appearance | Thick liquid | Thick liquid | Thick liquid |
| 3 | Ph | 5.49 ± 0.38 | 5.48 ± 0.36 | 5.57 ± 0.37 |
| 4 | Foam volume (mL) | 149.71 ± 0.46 | 149.59 ± 0.83 | 147.81 ± 0.29 |
| 5 | Dirt dispersion | None | None | None |
| 6 | Viscosity (cps) | 8868.61 ± 0.30 | 8869.70 ± 0.67 | 8868.94 ± 0.98 |
| 7 | Surface tension (dynes/cm) | 31.44 ± 0.48 | 32.18 ± 0.69 | 33.07 ± 0.63 |

## CONCLUSION

Nanotechnology is the new emerging technology in the field of drug discovery. Nanoparticles are smaller in size and can be used for targeting the infected pathological areas without the attachment of a specific ligand. An extensive research is going on in the area of novel drug delivery and for plant extracts and phytoconstituents. Hence there is a great potential in the development of nano phytomedicines. The therapeutic effects of herbal drugs are enhanced by incorporating the drug extract or phytoconstituents into novel drug delivery vesicles at a reduced dose. The nanotechnology has also adopted in the cosmetic formulations in order to overcome the drawbacks of conventional dosage forms.

A novel based herbal anti-dandruff shampoo was developed by incorporating herbal drug extract into solid lipid nanoparticles. The nanocarriers possess a greater capacity to protect the encapsulated drug molecules from degradation and transport deeply into the skin. By adopting this technique, it is possible to control the occurrence of dandruff formation. Hence this novel approach is found to be very effective for the treatment of dandruff.

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