

FORMULATION AND EVALUATION OF POLYHERBAL SHAMPOO

Kanchan B. Ghatge*, Satish V. Kilaje, Vaishnavi Heralge, Swati Magdum and Tejaswini Yamgar

Dr. J. J. Magdum Trust's, Dr. J. J. Magdum Pharmacy College, Jaysingpur, Tal. Shirol, Dist. Kolhapur - 416101, Maharashtra, India.



***Corresponding Author: Kanchan B. Ghatge**

Dr. J. J. Magdum Trust's, Dr. J. J. Magdum Pharmacy College, Jaysingpur, Tal. Shirol, Dist. Kolhapur - 416101, Maharashtra, India.

Article Received on 29/01/2025

Article Revised on 19/02/2025

Article Accepted on 09/03/2025

ABSTRACT

Shampoos are preparations use for the hair cleansing and removal of the dirt grease from the hair shaft and scalp. The aim of the present investigation was to formulate and evaluate herbal shampoo containing natural ingredients with an emphasis on safety and efficacy, which will avoid the risk posed by chemical ingredients. This study set out to design and assess the effectiveness of an herbal shampoo made from all-natural ingredients. The market offers a various options when it comes to Synthetic Shampoos. Shampoo's pre-mixed ingredients often include pharmaceutical medications. Several adverse responses to these drugs have been documented, including hair loss, severe scaling, discomfort, Scratching, nausea, and headache. The primary goal of this research was to replace potentially dangerous synthetic components in shampoo composition with benign natural ones. It gets rid of buildup like sebum and dandruff while also making hair thicker and darker. By using herbal natural extracts of Ocimum sanctum (Tulsi), Azadirachta Indica (Neem), Acacia Concinna (Shikakkai), Hibiscus Rosasinensis (Hibiscus), Murraya koenigii (Curry Leaves), Sapindus Mukorossi (Reetha), Aloe Barbadensis Miller (Aloe Vera), Flaxseed, Rice water; we can reduce the side effects of Synthetic Shampoo. Herbal shampoo is preferable to synthetic shampoo since it uses natural extracts from herbs to lessen the risk of allergic reactions.

1. INTRODUCTION

Mammal skin is typically covered in hairs. which developed from the outer layer of an embryonic cell. Main purpose of hair is facet of look in human. Alopecia, often known as baldness, is a condition in which an individual experiences a localized loss of hair. Over 40% of males over the age of 30 have hair loss, and a sizable proportion of women experience it as well. Loss of Hair.^[1] Scarring, illness, infection, decreased blood flow in the scalp's capillaries, and sensitivity to androgen are only some of the causes of hair loss among many others. Although a thorough history is essential in cases of alopecia. Paying close attention to how the hair loss manifests itself and performing a few basic tests can swiftly restrict the possible diagnosis.^[2] It also describes how the herbal medicine works to either stop hair loss or stimulate new growth. Hair care has become increasingly important in modern times as individuals worry about the effects of pollution on their strands. Split ends, roughness, slowed hair development, dullness, and hair loss are just some of the negative impacts of pollution on hair.^[3,4]

1.1 Hair problems

- **Hair loss:** Numerous causes, such as emotional or physical stress, pharmaceuticals, hormonal changes, and even some hair care products, have been linked to hair thinning and loss.

- **Oily hair/greasy hair:** An overactive sebaceous gland on the scalp is to blame for oily hair. Sebaceous glands "work overtime," resulting in an overabundance of sebum and oil.

- **Dandruff:** Dandruff, a benign scalp ailment, can cause itching and flaking and, in extreme cases, hair loss. It's a scaly buildup that gets stuck in hair follicle.

- **Dry hair:** Protein insufficiency is root cause of dry hair. Dry hair can also be caused by menopause, anemia, hormonal imbalance, or even the birth control pill.

- **Spilt ends:** Drying out the hair's ends, along with exposure to harsh climates, is a leading cause of split ends. Split ends can be caused by chemical hair products and styling methods like straightening and curling.^[5,6]

Shampoo addresses all of these issues, however the chemical components of synthetic shampoos can be damaging to the hair. There are large range of synthetic shampoos available on the market with varied purposes. However, using synthetic shampoos leads to dryness and keratin loss in the hair and scalp. However, repeated use of synthetic shampoos containing surfactants (synthetic detergents) can lead to serious adverse effects such as discomfort of eyes and scalp, hair loss, and dry hair.^[7] Because they use time-tested, historically safe

ingredients, herbal shampoos have gained popularity as a replacement to synthetic shampoo. Many of the herbs are utilized in herbal shampoos due to their purported positive effects on hair.

The goals of study are to

- (1) Produce herbal shampoo.
- (2) Lessen the negative effects of shampoo's chemical creation.
- (3) Make the hair feel smoother.
- (4) Make the hair a darker shade of brown.

Purpose of this investigation was to manufacture herbal shampoo and to assess and compare physicochemical properties of commercially available herbal and synthetic shampoos in an effort to identify a product that offers the advantages of both types. Human hair contributes greatly to our physical attractiveness. Most people have noticed a variety of hair issues, including thinning hair, a lack of hair volume, premature greying, a lack of conditioning, hair loss, etc.

1.2 Shampoo

Shampoos are the cosmetics preparations meant for cleansing the hair by removal of the dirt grease from the hair shaft and scalp. The aim of the present investigation was to formulate and evaluate herbal shampoo containing natural ingredients with an emphasis on safety and efficacy, which will avoid the risk posed by chemical ingredients.

Types of shampoos

- Lotion
- Powder
- Liquid

- Aerosol
- Conditioning
- Cream
- Anti. dandruff
- Traditional
- Herbal
- Specialized
- Jelly
- Solid.



1.3 Functions of Shampoo







- It must eliminate dirt or soil thoroughly and effectively.
- It should thoroughly clean hair.
- It should generate enough foam to ease the user.
- Washing with water should effortlessly remove it.
- It should leave a pleasant fragrance in the hair without irritating the skin or eyes.





1.4 Ideal characteristic of herbal shampoo

- It's good for your hair, but too much of it will make it look unclean.
- Should get rid of the dust and extra sebum totally and effectively.
- Hair should be washed well.
- It should make a good amount of foam.
- Rinsing your hair with water should make it easy to get rid of the shampoo.
- Should keep the hair from drying out, be soft, shiny, and easy to style.
- This product should give your hair a nice scent.
- Shouldn't make the hands rough and dry.
- Shouldn't hurt you or make your eyes or skin itch.^[8,9]

2. Herbs commonly used in herbal shampoo

| | |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|
| <p>Murraya koenigii Synonym – Sweet neem leaves Biological Source – It consist of dried leaves of plant <i>Bergera koenigii</i> L. Family - Rutaceae. Chemical Constituents – Vitamins – C, B, E, Proteins, antioxidants. Uses – Straighten the hair roots , Treat dandruff, Darkens the hair colour. Promotes hair growth</p> |  <p style="text-align: center;">Fig. No. 1</p> |
| <p>Hibiscus Synonym - Jaswand, shoe flower Biological Source – It consist of flowers of plant <i>Hibiscus rosa sinensis</i> L. Family – Malvaceae Chemical Constituents – Vitamins, quercetin-3-diglucoside, kaemferol-3- xylosylglucoside, 5 -diglucoside, beta-sitosterol, iron, beta carotene, calcium, tannin, flavonides, saponins Uses – Stimulates hair growth, Prevent premature greying.</p> |  <p style="text-align: center;">Fig. No. 2</p> |

| | |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------|
| <p>3. Azadirachta Indica Synonym – Kadunimb, Margosa Biological Source – Fresh leaves or dried leaves powder of plant <i>Azadirachta indica</i> A. Juss., Family- Meliaceae. Chemical Constituents – Simasterol, not less than 1% w/w of Rutin Uses – Cures dandruff, Relives drying and itching, Antilice property, Prevents hair loss.</p> |  <p>Fig. No. 3</p> |
| <p>4. Aloevera Synonym – Aloe , Musabber , Kumari Biological Source – Aloes is the dried juice of the leaves of <i>Aloevera</i> (L.) Burm. Family- Asphodelaceae. Chemical Constituents – anthraquinone glycosides, aloin Uses – Deep clean oily hairs, Strengthens and repairs hair strands, Promote hair growth, Make hair smooth and silky</p> |  <p>Fig. No. 4</p> |
| <p>Holy Basil Synonym – Sacred basil, Holy Basil Biological Source – Tulsi Consist of fresh and dried leaves of <i>Ocimum tenuiflorum</i> Linn. Family- Lamiaceae Chemical Constituents – volatile oil, 70 % eugenol, carvacrol and eugenol- methyl –ether, caryophyllin Uses – Helps to maintain moisture in scalp, improve blood circulation, treating premature graying of hairs. Reduce the hair fall, it shows antibacterial activity</p> |  <p>Fig. No. 5</p> |
| <p>Green tea Biological Source – It consist of dried leaves of plant <i>Camellia sinensis</i> L. Family- Theaceae Chemical Constituents – Catechin, Epicatechin, Gallocatechin, Epigallocatechin, Epicatechin Gallate, Epigallocatechin Gallate and (+)-Gallocatechin Gallate, Phenols, Alkaloids-Caffeine, Methylxanthine, Lignin Carotenoids. Uses – Boost hair colour, prevents hair fall, treat dandruff</p> |  <p>Fig. No. 6</p> |
| <p>Soap Nut (Reetha) Synonym – Soap nut, Aritha Biological Source – It consist of the dried as well as fresh fruits of the species <i>Sapindus mukorossi</i> Family – Sapindaceae Chemical Constituents – triterpenoid saponins named Sapindoside A&B, fruits also contain sesquiterpenoidal glycosides and six different fatty ester of tetracyclic triterpenoids. Uses – Cleanses the skin of oily secretion and is even used as a cleanser for washing hair</p> |  <p>Fig. No. 7</p> |
| <p>Shikekai Synonym – Vimala, bhuriphena Biological Source – It consist of dried fruits of plant <i>Acacia concinna</i>, Family - Fabaceae Chemical Constituents – Citric acid, tartaric acid, lactose Uses – Cleanses hair , prevents split ends , crubs hair loss</p> |  <p>Fig. No. 8</p> |

| | |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|
| <p>Rice Water Synonym – Rice Biological Source – It consists of embryo and endosperm of the seeds of <i>Oryza sativa</i> . Family – Graminae / Poaceae Chemical Constituents – The rice grain constitutes 12% water, 75–80% starch(carbohydrate), 7% protein, 3% fat and 3% fibres. Uses – Promotes hair growth, helps to remove dandruff, gives shine and luster to the hairs.</p> |  <p>Fig. No. 9</p> |
| <p>Onion Seeds Synonym - Kalonji, Black cumin Biological Source – It consists of dried seeds of <i>Alium cepa</i> Family- Amaryllidaceae Chemical Constituents - Triterpenoids, Steroids, Glycosides, Saponins, Alkaloids, Flavonoids, Tannins, Proteins, Free amino acids, Carbohydrate and Vitamin C Uses – Anti-allergic, Antioxidant and Anti-inflammatory activities</p> |  <p>Fig. No. 10</p> |
| <p>11. Lemon Juice Synonym –Limonis Fructus, Limonis Cortex Biological Source – Lemon is the fruit of <i>Citrus limon</i> (L.) Burm., Family- Rutaceae Chemical Constituents – ascorbic acid, carbohydrates, Fat , zinc, calcium, potassium, magnesium, iron, phosphorus, protein, riboflavin, thiamine, pantothenic acid (B5) Uses – Shampoos often include citric fruits, like lemon, for their fragrant smell and ability to adjust the scalp to a 5.5 pH. this helps to: maintain neutrality, prevent any adverse reactions and inflammation on the skin.</p> |  <p>Fig. No. 11</p> |
| <p>Flax Seeds Synonym- Flax seed, Alsi Biological Source- Linseed is the dried , ripe seed of <i>Linum usitatissimum</i> Linn. Family- Linaceae Chemical Constituents - Fixed oil (30-40%), mucilage (6-10%), protein (25%), Unsaturated acid (linolenic acid, linoleic acid ,oleic acid), Saturated acid (myristic, stearic, palmitic). Uses- Promotes quicker and longer hair growth, gives shine and luster, removes dandruff, nourishes hairs.^[10, 11, 12]</p> |  <p>Fig. No. 12</p> |

3. MATERIALS AND METHODS

3.1 Collection of plant materials

The herbal shampoo was formulated using the following natural ingredients were Flowers of Hibiscus Rosasinensis (Hibiscus), Ocimum Sanctum (Tulsi), Azadirachta Indica (Neem), Murraya koenigii (Curry Leaves), Sapindus Mukorossi (Reetha), Acacia Concinna (Shikakkai), Onion seeds and Aloe Barbadensis Miller (Aloevera). The Materials used in the study were collected from the nearby areas, college medicinal garden and were properly authenticated in the Department of Botany at Jaysingpur college, Jaysingpur. Drying: All the ingredients required for shampoo preparation were shade dried for 5 days. Size Reduction: Dried materials are subjected to size reduction by using hand driven mixer. Sieving: Pass the powdered material through the sieve number 80 to get uniform particle size.

3.2 Formulation of Herbal Gel Shampoo

3.2.1. Preparation of Extracts

Take required amount of each powdered plant materials, namely Flowers of *Hibiscus Rosa sinensis* (Hibiscus),

Ocimum tenuiflorum (Tulsi), *Azadirachta indica* (Neem), *Bergera koenigii* (Curry Leaves), *Sapindus mukorossi* (Reetha), *Acacia concinna* (Shikakkai), *Camellia sinensis* (Green tea), *Allium cepa* (Onion seeds) and Aloe Vera were mixed together. The powdered material was extracted with distilled water by boiling for 4 hours.

Preparation of Flaxseed solution

Add the flaxseeds to the sufficient quantity of water. Boil this water for around 10 minutes and keep stirring to avoid the flaxseeds from sticking to the base of the beaker. Turn the burner off when you achieve a gel like texture, not too dense but not too thin. Let the gel cool down for about an hour while it thickens. Squeeze the gel using a clean muslin cloth.^[14]

3.2.3. Preparation of Rice water

Take a cup of rice in a clean bowl. Add some amount of water to the rice containing bowl. Then wash the rice until the dirt and impurities are removed. Again add sufficient amount of water to rice and cover the bowl with heavy lid. Keep the bowl aside at room temperature

for a 2 to 3 days. Later, collect the fermented rice water and transfer into a clean glass container.^[15]

Formulation Table

Table 1: Weight of powder for herbal extract.

| Ingredients | F1 (gm) | F2 (gm) | F3 (gm) | F4 (gm) |
|--------------|---------|---------|---------|---------|
| Hibiscus | 5 | 6 | 8 | 7 |
| Neem | 5 | 6 | 8 | 7 |
| Onion seeds | 1.5 | 2 | 3.5 | 1.5 |
| Green tea | 2.5 | 3 | 3.5 | 2.5 |
| Curry leaves | 1.25 | 1.5 | 2 | 1 |
| Tulsi | 2 | 2.5 | 3 | 1.5 |
| Aloe | 5 | 6 | 8 | 7 |
| Reetha | 10 | 13 | 15 | 8 |
| Shikekai | 10 | 10 | 13 | 10 |

Table 2: Formula for Shampoo.

| | | | | |
|-------------------------|-------|-------|-------|-------|
| Herbal Extract | 20 ml | 20 ml | 20 ml | 20 ml |
| Lemon juice | q.s. | q.s. | q.s. | q.s. |
| Rice water | q.s. | q.s. | q.s. | q.s. |
| Flax seeds | q.s. | q.s. | q.s. | q.s. |
| Sodium chloride (0.1 M) | 5 ml | 5 ml | 5 ml | 5 ml |
| Lavender Essential oil | q.s. | q.s. | q.s. | q.s. |

3.2. Procedure for preparation of herbal shampoo

- Take about 20 ml of extract.
- Add 0.1M Sodium chloride as a preservative.
- Add required quantity of rice water and flaxseed solution to make the gel like consistency of shampoo.
- Add 1-2 ml of lemon juice to adjust the pH.

4. Evaluation Test

1) Physical appearance/visual inspection

The formulations prepared were evaluated for its clarity, colour, odour, and texture.^[16]

2) Determination of pH

The pH of 10% shampoo solution in distilled water was determined at room temperature 25°C.^[17]

3) Determination of percent solid content

Take and weigh clean and dry evaporating dish and add about 4 grams of shampoo to the evaporating dish. The dish along with the shampoo was weighed again. The exact weight of the shampoo was calculated only and the evaporating dish with shampoo was placed on the hot plate until the liquid portion was evaporated. The weight of the shampoo only (solids) after drying was calculated.^[16]

Formula for calculation % solid content –

$$\% \text{ solid content} = \frac{\text{Weight of dry sample} \times 100}{\text{Weight of wet sample}}$$

4) Dirt dispersion test

Two drops of shampoo were added in a large test tube contain 10 ml of distilled water. 1 drop of ink was added; the test tube was stoppered and shake it ten times. The

amount of ink in the foam was estimated as None, Light, Moderate, or Heavy.^[17]

5) Foaming ability and foam stability

Cylinder shake method was used for determining foaming ability. 50 ml of the 1% shampoo solution was put into a 250 ml graduated cylinder and cover the cylinder with hand and shaken for 10 times. The volume of the foam appeared due to shaking is measured after every 1 minute consecutively for 4 minutes.^[18]

6) Wetting time

The canvas was cut into 1-inch diameter discs having an average weight of 0.44g. The disc was floated on the surface of shampoo solution of 1% w/v and the stopwatch started. The time required for the disc to begin to sink was measured accurately and noted as wetting time.^[18]

5. Phytochemical Screening

The aqueous herbal shampoo extract was subjected to phytochemical analysis which was carried out by following the procedures for Tannins, Saponins, Flavonoids, Alkaloids, Proteins, and Phenols were estimated by ensuring the Successive methods.^[19,20,21]

1. Tannins

1 ml of aqueous herbal shampoo extract and 2-3 drops of 0.1% ferric chloride were combined and checked for the appearance of blue/black/brownish green color.

2. Saponins

1 ml of extract and 2ml of water were added, shaken continuously and checked for foam appearance.

3. Flavonoids

➤ Alkaline reagent test-

To the 2 ml of extract add 2 ml of sodium hydroxide and checked for the appearance of yellow color.

➤ Lead acetate test-

To the 2 ml of extract add 2 ml of lead acetate and checked for the appearance of yellow color.

4. Alkaloids

➤ Dragendroff's test

To the 1 ml of extract add 2-3 drops of Dragendroff's reagent checked for the formation of orange red colored precipitate.

➤ Mayer's test

To the 1 ml of extract add 2-3 drops of Mayer's reagent checked for the formation of yellow red colored precipitate.

5. Carbohydrate

➤ Molisch's test

Take 2 ml of extract, add 2-3 drops of Molisch's reagent and Conc. Sulphuric acid. Checked for the formation of yellow colored precipitate.

➤ Fehling's test

To the 1 ml of extract, add 2 ml Fehling's A and Fehling's B solution. Heat the solution and observe for the formation of red colored precipitate.

6. Phenols

➤ Ferric chloride test-

Take 2 ml of extract, add 3-4 drops of ferric chloride reagent. Observe the formation of bluish black color.

7. Protein and amino acid

➤ Ninhydrin test-

To the 1 ml of extract, add 0.25 % Ninhydrin reagent. Heat the solution and observe the formation of blue color.

6. Thin Layer Chromatography

➤ For tannin

- TLC is performed using Silica Gel G plates.
- Mobile phase used for detection of tannin is n-butanol: Glacial acetic acid: water (4:1:5).
- The chromatogram is developed by using Iodine chamber.

➤ For Saponin

- TLC is performed using Silica Gel G plates.
- Mobile phase used for detection of saponin is Chloroform: Methanol: water (65:35:10)
- The chromatogram is developed by using Iodine chamber.^[22]

7. Phytochemical Screening

➤ Total phenolic content

Preparation of Standard Gallic Acid for Calibration Curve

Total phenolic contents (TPC) in the fruits, seeds and bark extracts were determined by Folin-Ciocalteu

colorimetric method as described by Singleton et al. with some modifications. Standard Gallic acid solution was prepared by dissolving 10 mg of it in 10 mL of methanol (1 mg/mL). Various concentrations of Gallic acid solutions in methanol (25, 50, 75, and 100 µg/mL) were prepared from the standard solution. To each concentration, 5 mL of 10% Folin-Ciocalteu reagent (FCR) and 4 mL of 7% Na₂CO₃ were added making a final volume of 10 mL. Thus, the obtained blue colored mixture was shaken well and incubated for 30 min at 40°C in a water bath. Then, the absorbance was measured at 760 nm against blank. The FCR reagent oxidizes phenols in plant extracts and changes into the dark blue color, which is then measured by UV-visible spectrophotometer. All the experiments were carried out in triplicates, and the average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve.^[23]

Preparation of Samples for Total Phenolic Content:

Various concentrations of the extracts (25, 50, 75, and 100 µg/mL) were prepared. The procedure as described for standard gallic acid was followed, and absorbance for each concentration of the extracts was recorded. The samples were prepared in triplicate for each analysis, and the average value of absorbance was used to plot the calibration curve to determine the level of phenolics in the extracts. Total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The total phenolic contents in all the samples were calculated by the using the formula:

$$C = c \cdot V/m$$

Where, C = total phenolic content mg GAE/g dry extract.

c = concentration of Gallic acid obtained from calibration curve in mg/mL.

V = volume of extract in mL.

m = mass of extract in gram.

8. Antidandruff activity

Method of analysis

Two general method usually employed; One is the cup-plate method [Agar well diffusion method)-The agar cup plate method depends upon diffusion of the antibiotic from a vertical agar [CUP] Cylinder through a solidified agar layer on a Petri dish. Sterile Agar is inoculated by suspension of the microbial inoculum. Then a hole with diameter of 6 to 8 mm is punched aseptically with a sterile cork borer or a tip, and then of the antimicrobial solution at desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain entirely in a zone around the cylinder containing a solution of the substance to be tested.^[24,25,26]

Media and reagents preparation

Antibiotic Assay Medium No. 19 (pH is 6.1 ± 0.2).

Table 3: List of ingredients.

| Ingredients | Quantity taken |
|-----------------|----------------|
| Peptone | 9.4 |
| Yeast Extract | 4.7 g |
| Beef Extract | 2.4 g |
| Sodium chloride | 10 g |
| Dextrose | 10 g |
| Agar | 23.5 g |
| Water | 1000 mL |

Prepared of medium Antibiotic Assay Medium No. 19 in 600 ml of purified water, heat boiling to dissolve the medium completely check the pH of media. If required add sufficient 1 M sodium hydroxide or 1 M hydrochloric acid, distributed in 200 ml flasks as required so that after sterilization as quantity as per required for analysis., sterilized by autoclaving it at 15 lbs pressure (121°C) for 15 min.

Preparation of the sample solution

Use directly 100 µl and 200 µl to inoculate.

Preparation of Test organism and suspension

- Test organisms- *Malassezia furfur* ATCC no 12078
- Stock culture *Malassezia furfur* ATCC no 12078

Streak a loopful of suspension ATCC. 12078 on two slants of pre incubated sabroud dextrose agar. Incubate the slants at 30-35°C for 24 hours in an incubator After incubation pick up the growth from incubated slant & inoculate in 3 ml of saline solution & vortex to prepare the uniform suspension. Adjust the O.D. of culture to approx. 60-70 % OD at 530 nm using sterile saline and calorimeter. After adjusting O.D. store the test organism in refrigeration at 2-8°C.

Plate Preparation for analysis

After the suspension is prepared, use each 2 ml of culture suspension of *Malassezia furfur* is to inoculate separately

in 200 ml of sterile molten and cooled medium at 40°C - 45°C Antibiotic Assay Medium No. 19. 15-20 ml of Sterilized agar medium is poured into a sterile Petri plate with the help of sterile measuring cylinder give a depth of 3 to 4 mm. Allow to cool at room temperature by placing the dishes or plates on a level surface. Keep plates in refrigerator for 15 to 20 minute for hardening. Ensure that the layers of medium are uniform in thickness. Make 4-5 agar cups on each plate using 8-10 mm SS borer. Label the plates for sample, standard and negative control samples and analysis details.

Analysis

The volume of solution added to each cylinder or cavity must be uniform and sufficient almost to fill the holes when these are used. Add 100 µl 1mg/ml Solution A to agar cup labeled as STD. Sample directly used as 100 µl and 200 µl to wells. Leave the dishes or plates standing for 15-20 min. at 2-8°C or as appropriate, as a period of pre- incubation diffusion to minimize the effects of variation in time between the applications of the different solutions. Incubate them for about 24-48 hours at the temperature 30-35°C for bacteria and 20-25°C for yeast and mould. After completion of incubation accurately measure the diameters or areas of the circular inhibition zones and record the results.

9. RESULT AND DISCUSSION

• For Herbal Gel Shampoo

1) Physical Appearance

Table 4: Physical Appearance of herbal gel shampoo

| Name of test | F1 | F2 | F3 | F4 | Marketed shampoo (Indulekha) |
|--------------|------------|------------|------------|------------|------------------------------|
| Clarity | Clear | Clear | Clear | Clear | Clear |
| Color | Dark brown | Dark brown | Dark brown | Dark brown | Dark brown |
| Odour | Pleasant | Pleasant | Pleasant | Pleasant | Strong |
| Texture | Gel | Gel | Gel | Gel | Gel |

**Fig. No. 5.1: Herbal Shampoo.**

Shampoos, similar to other cosmetic products, should have an attractive physical appearance. Table-7.1 shows the evaluation of physical characteristics such as clarity, color, odor, and texture for both the formulated herbal shampoo and the marketed herbal shampoos. Our shampoo variants, labeled F1, F2, and F3, F4 are clear, dark brown and possess a pleasant odour. Marketed shampoos showed no significant differences in above

characteristics when compared to the formulated shampoo, except in terms of odour.

2) pH

Table 5: pH of herbal gel shampoo.

| Name of test | F1 | F2 | F3 | F4 | Marketed shampoo |
|--------------|------|------|------|------|------------------|
| pH | 7.65 | 7.64 | 7.78 | 7.66 | 6.43 |

The pH value of formulated shampoo and Marketed shampoo are within the preferred range of 5- 7 were shown in the table No.5.

3) Solid content

Table 6: Solid content of herbal gel shampoo.

| Name of test | F1 | F2 | F3 | F4 | Marketed shampoo |
|---------------|-----|-----|-----|-----|------------------|
| Solid content | 93% | 96% | 95% | 84% | 88% |

The percent solid content of formulated F3, F4 and Marketed shampoo are within the range of 20 to 30% and expected to easily wash out. The F1, F2 are does not

passes the test for solid content which is shown in Table: 6.

4) Wetting time

Table 7: Wetting time of herbal gel shampoo.

| Name of test | F1 | F2 | F3 | F4 | Marketed shampoo |
|--------------|-----------|-----------|-----------|-----------|------------------|
| Wetting time | 1.42 min. | 1.46 min. | 1.48 min. | 2.01 min. | 2.10 min. |

The normal wetting time range is 1-2 min. the F1, F2 & F3 are passes the test and F4 and marketed shampoo is not passes the test. Is shown in Table No. 7.

5) Dirt dispersion

Table 8: Dirt dispersion of herbal gel shampoo.

| Name of test | F1 | F2 | F3 | F4 | Marketed shampoo |
|-----------------|-----------------------------------|-------|-------|-------|------------------|
| | Amount of ink present in the foam | | | | |
| Dirt dispersion | Light | Light | Light | Light | Light |

Dirt dispersion test is essential factor in determining how thoroughly it cleans. The ink was concentrated in water portion of every shampoo, ensuring their adequate cleansing capacity and practically usefulness. The F1,

F2, F3, F4 and marketed shampoo shows light in ink concentration in foam. Hence all are passes this test, which are shown in Table No. 8.

6) Foaming ability and stability

Table 9: Foaming ability and stability of herbal gel shampoo.

| Name of test | F1 | F2 | F3 | F4 | Marketed shampoo |
|-------------------------------|------|-------|------|-------|------------------|
| Foaming ability and stability | 8 ml | 10 ml | 7 ml | 10 ml | 30 ml |

From the Foaming ability and stability test (Table 9), we found that formulated as well as marketed shampoo produces foam.

7) Phytochemical screening

Table 10: Phytochemical Screening.

| Sr. No. | Test | Observation | Result |
|---------|-----------------------------|---------------------|---------|
| 1 | Tannin | Green Color | Present |
| 2 | Saponin | Foam Formation | Present |
| 3 | Flavonoid Lead acetate test | Yellow Color | Present |
| | Alkaline reagent test | Yellow Color | Present |
| 4 | Alkaloid Dragendroff's test | Reddish Brown Color | Present |

| | | | |
|---|---------------------------------------|-------------------------------------|---------|
| | Mayer's test | Cream Coloration | Present |
| 5 | Carbohydrate Molisch's test | Violet Colored Ring at the Junction | Present |
| | Fehling test | Orange Red Precipitation | Present |
| 6 | Phenol Ferric chloride test | Bluish Black Color | Present |
| 7 | Protein and amino acid Ninhydrin test | Blue Color | Present |

From the above Table 10. it was found that extract of herbal shampoo contains Tannin, Saponin, Flavonoid, Alkaloid, Carbohydrate, Phenol, Protein and amino acid.

8) Analytical Studies

Thin layer chromatography.

a) TLC for Saponin

Observation

Distance travelled by solute= 4.7 cm.

Distance travelled by solvent= 5.8 cm.

Calculation:

Formula:

RF value = Distance travelled by solute

Distance travelled by solvent

= 4.7 / 5.8

= 0.81



Fig. No. 5.2: TLC for saponin.

b) TLC for Tannin

Observation

Distance travelled by solute= 4.0 cm.

Distance travelled by solvent= 5.5 cm.

Calculation

Formula:

RF value = Distance travelled by solute

Distance travelled by solvent

= 4.0 / 5.5

= 0.72.



Fig. No. 5.3: TLC for Tannin.

9) Determination of Phenolic Content

Observation Table 11.

| Sr. No. | Concentration | absorbance of Standard Gallic Acid | Absorbance of Extract |
|---------|---------------|------------------------------------|-----------------------|
| 1 | 25 | 0.493 | 1.52 |
| 2 | 50 | 0.262 | 1.964 |
| 3 | 75 | 0.738 | 2.321 |
| 4 | 100 | 0.881 | 2.573 |

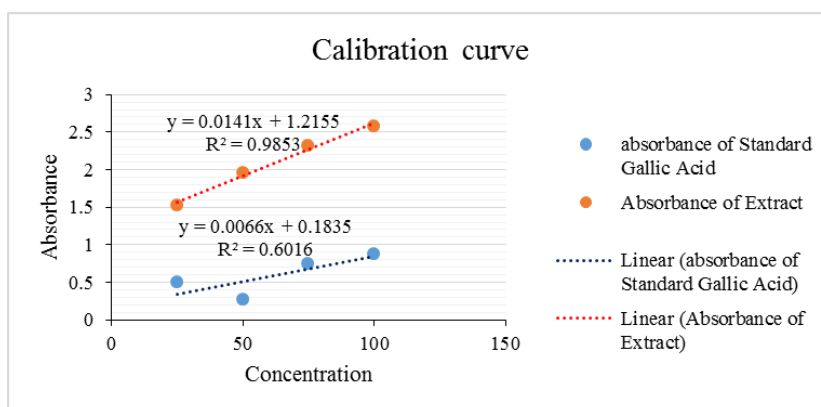
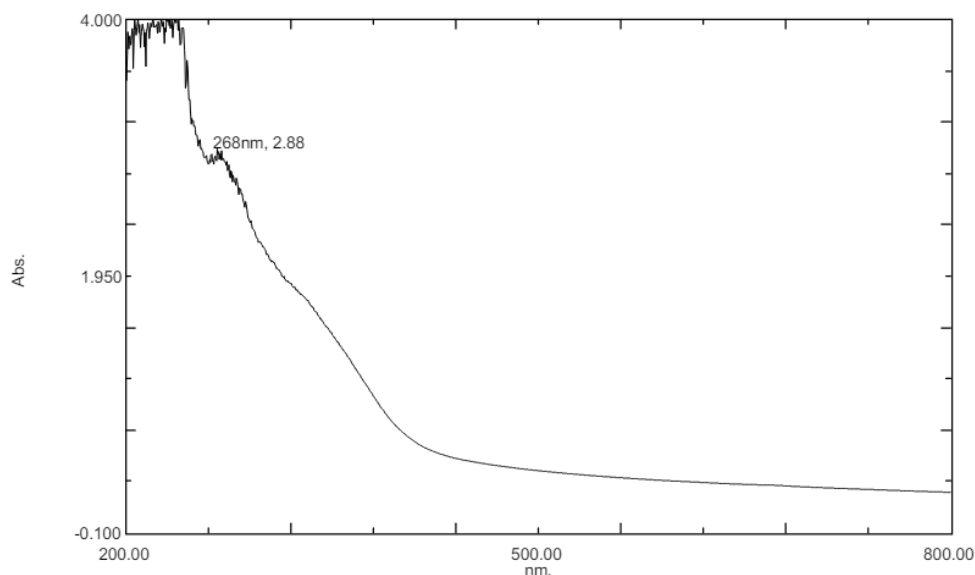


Fig. No. 5.4: Calibration Curve.

Calculation

Equation from graph,

| | |
|--------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| For Gallic Acid, $y = 0.0141x + 1.2155$ $R^2 = 0.9853$ | $y = mx + c$ $x = \frac{1.52 - 0.1835}{0.0066}$ $= 0.2025 \text{ mg/ml}$ $C = xv/m$ $C = 30.68 \text{ GAE/gm}$ |
| For Extract, $y = 0.0066x + 0.1835$ $R^2 = 0.6016$ | |

UV Analysis of Herbal Shampoo**Fig. No. 5.5: UV-visible spectrophotometer.**

| Sr. no. | Wavelength | Absorbance |
|---------|------------|------------|
| 1 | 268 | 2.883 |

UV-VIS spectroscopy helps in identifying the presence of different phytochemicals in the extract by detecting characteristic peaks in the wavelength range of 190-500nm.

The UV absorption peaks at the value of 268 nm, which indicates the presence of phenolic and flavonoid content in the extract.

10) Antidandruff Activity

Observation

Dose of compound = 100 μ l Dose of standard = 1 mg/ml
= 200 μ l

**Fig. No. 5.6: Zone of Inhibition.****Table 12: Observation Table for Antidandruff Activity.**

| Sr. No. | Sample | Concentration | Zone of Inhibition (mm) <i>Malassezia furfur</i> |
|---------|----------------------|---------------|--------------------------------------------------|
| 1 | Standard Fluconazole | 1mg/ml | 30 |
| 2 | Sample (F4) | 100 μ l | 15 |
| | | 200 μ l | 18 |

During the study it has been found that sample showed Moderate inhibiting the growth of micro-organisms against *Malassezia furfur*.

10. CONCLUSION

The purpose of this analysis was to highlight the steps necessary to make an herbal shampoo that stops hair loss while also boosting growth and tensile strength. The goal

of this formulation was to create a formula for a shampoo that would be both stable and effective, and that's exactly what it did. We can lessen the negative effects of shampoo by utilizing extracts from natural herbs. Safer than synthetic shampoo is herbal shampoo. Herbal shampoo was made with the aqueous extract of medicinal plants that are often used for cleansing hair historically. Instead of using synthetic ingredients, the researchers in this study rely on natural plant extracts like shikakai and flax seed, which have antidandruff, antibacterial, antioxidant, and antifungal qualities. Formulators have a significant responsibility to alter the public's understanding of what constitutes a high-quality shampoo. Hence the developed herbal shampoo was safe and effective for use and aids in minimizing hair fall.

REFERENCES

1. Andrew GM. The Control of Hair Growth: An Overview. *J Inves Dermatol*, 1993; 10: 523-27.
2. Kumar N, Singh S, Manvi. Hair Growth Activity of *Trichosanthes dioica* R. Leaves. *J Pharmacog Phytochem*, 2011; 3: 30.
3. Manohar D. Kengar, Ganesh B. Vambhurkar, Akshata G. Gavade, Asha M. Jagtap, Indrayani D. Raut, Formulation and Evaluation of Poyherbal shampoo, *Research Journal of Topical and cosmetic science*, 2018; 9: 44-50.
4. Prabhat Dessai, Shiny Phatarpekar, Formulation and evolution of herbal shampoo formulations and to compare formulated shampoo with marketed shampoos, *World journal of pharmacy and pharmaceutical science*, 2028; 5(9): 1467-1477.
5. Ameya Lanjewar^{1,2}, Soni Maurya³, Devender Sharma^{4,5}, Anchal Gaur, Review on Hair Problem and its Solution, *Journal of Drug Delivery & Therapeutics*, 2020; 10(3-s): 322-329.
6. Angela M. Pagarana, Janelle Marie Erika L. Dematingcala, Divine Grace E. Serafina, Alona Mae A. Dela Torrea, Rio Marie M. Timarioa, Joseph Mari Baguilot Querequinciab, An Overview of Herbal Ingredients with Anti-dandruff Activity in Shampoo Formulations, *International Journal of Research Publication and Reviews*, January 2023; 4(1): 795-812.
7. Yadav SK, Gupta SK, Prabha S. Hair growth activity of *Nardostachys jatamansi* and *Cyperus rotundus* rhizomes extract on chemotherapy induced alopecia. *Int J Drug Dis Herbal Res.*, 2011; 1: 52-4.
8. Palwe Vimal Devidas, Dr. L. D. Hingne Formulation and Evaluation of Herbal Shampoo from Piper Betel and Psidium Guajava Leaves *International Journal for Research in Applied Science & Engineering Technology (IJASET)* ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.538 Volume 10 Issue VI June 2022.
9. Ashwini Sukhdev Pundkar* and Sujata P. Ingale, formulation and evaluation of herbal liquid shampoo-I, *World Journal of Pharmaceutical Research*, 9(5): 901-911. Research Article ISSN 2277- 7105
10. Priti S. Narkhedkar, V. D. Nawale, P. S. Narwade "preparation and evaluation of herbal hair shampoo", 2023; 12(Special Issue 4): 10326.
11. Aniket Pawar, Rakesh Pomnar, Mahesh Sawant "the formulation and evaluation of herbal shampoo" (*ijcrt*) Volume 10, Issue 7 July 2022; 257-263.
12. Vineetha K, Vindhya V S, Vishranth M B."herbal shampoo: benefits, preparation and evaluation" *Journal of Xi'an Shiyou University, NSE, VOLUME 17; ISSUE 09* 646 -660.
13. Deepak Somavanshi, Chandrashekhar Patil, Ganesh Sonawane, Kajal Pansare and Mohammed Saad, formulation evaluation of the multifunctional paraben free herbal shampoo from household herbs and comparison with the commercial shampoos, *International journal of pharmaceutical sciences and research*, Volume 14, Issue 5, 2483-2490.
14. M. Sankara Bhavani*, Shaik Mehamuda Jan, Kota Sandhya Rani, M.Srirekha, Formulation, Evaluation and Comparison of the Herbal Shampoo with Commercial Shampoos, *Int. J. Pharm. Sci. Rev. Res.*, 78(1), January – February, 2023; Article No. 07, 41-45.
15. Udaya Guttikonda , Yamini V, Padma Priya Ch, Sameera Sk, Naveen Ahmad Sd, Keerthana K, Formulation, evaluation and comparison of polyherbal antidandruff shampoo cum conditioner with marketed shampoo, *journal of pharma insights and research*.
16. Thakkar Krunali, Patel Dhara, D.B Meshram, Patel Mitesh, evaluation of standards of some selected shamppoo preparation, *world journal of pharmacy and pharmaceutical sciences*, Volume 2, Issue 5, 3622-3630. Research Article ISSN 2278 – 4357
17. Khaloud Al Badi, Shah A. Khan, Khaloud Al Badi, Shah A. Khan, Formulation, evaluation and comparison of the herbal shampoo with the commercial shampoos, *beni-suef university j ournal of basic and applied sciences* 3, 2014; 301-e305.
18. Ramadevi B and Gangarao Battu, A holistic approach for formulation and evaluation of poly herbal shampoos, *Journal of Pharmacognosy and Phytochemistry*, 2019; 8(2): 829-835.
19. U.S. Mahadeva Rao, Muhammad Abdurrazak, Khamsah Suryati Mohd, phytochemical screening, total flavonoid and phenolic content assays of various solvent extracts of tepal of *Musa paradisiaca*, *Malaysian Journal of Analytical Sciences*, 2016; 20(5): 1181 – 1190.
20. Sana Wahid, Samiyah Tasleem and Sajid Jahangir, phytochemical profiling of ethanolic flower extract of *hibiscus rosa-sinensis* and evaluation of its antioxidant potential, *World Journal of Pharmaceutical Research*, Volume 8, Issue 6, 161-168. Research Article ISSN 2277– 7105.
21. T. Deepika and C.M. Noorjahan, Phytochemical Screening and Antioxidant Property of Aqueous *Murraya Koenigii* (Curry Leaf) Extract, *International Journal of Research in Advent Technology*, Vol.7, No.2, February 2019. E-ISSN:

- 2321-9637.
22. Mrs. Pranali Kumbhalkar, Mrs. Gayatri chinchulkar, Mrs. Pranita Dhapke", Formulation and Evaluation of 1 % Shampoo for Antimicrobial Activity Containing Flavonoid Rich Fraction of Aerial Parts of *Cassia auriculata*" JETIR September, 2020; 7(9): 454 – 456.
 23. Phuyal N., P. Kumar Jha, P. Raturi P.P. and Rajbhandar S. Total Phenolic, Flavonoid Contents, and Antioxidant Activities of Fruit, Seed, and Bark Extracts of *Zanthoxylum armatum* DC Hindawie Scientific World Journal Volume 2020, Article ID 8780704.
 24. Hufford CD, Funderburk JM, Morgan JM, Robertson LW. Two antimicrobial alkaloids from heartwood of *Liriodendron tulipifera*. I.J.pharm. Sci., 1975; 64: 789-792.
 25. Umadevi S, Mohanta G P, Chelladurai V, Manna PK, Manavalan R. Antibacterial and antifungal activity of *Andrographis echinodes*. J. Nat. Remedies., 2003; 3: 185-188.
 26. Saifullah Khan and Gul Majid Khan, In vitro Antifungal activity of *Rhazya stricta*, Pak. J. Pharm. Sci., 2007; 20(4): 274-27.