Formulation of Anti- Dandruff Herbal Mask for Healthy Hair

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Abstract-
Background – Dandruff and hair loss are significant issues related to hair, and they cannot be completely resolved with chemical-based solutions. This study aimed to develop a poly herbal formulation for anti-dandruff care, ensuring its stability and quality.

Objective – The main objective of this research was to create an herbal hair formulation for combating dandruff and evaluate its various characteristics to determine its effectiveness.

Materials and methods – The herbal preparation was produced in the laboratory using specific proportions of herbal ingredients. The resulting mixture was then assessed based on several parameters.

Results – The obtained results were considered satisfactory for evaluating the herbal hair pack formulation. The outcomes from different aspects demonstrated the effectiveness of the flaxseed-based herbal hair pack formulation.

Conclusion – The formulation of the flaxseed-based herbal hair pack was successfully developed and evaluated based on various aspects. It provides a viable alternative without any adverse effects. The results can be utilized in the development of standardized pharmacopeia guidelines.

Key words- Malassezia, Dandruff, Formulation, Herbal,

1. INTRODUCTION

Human hair plays a significant role in social and sexual contamination, with notable distinctions between different ages and genders, in addition to its protective function. Many individuals encounter issues related to their scalp health, such as dandruff, folliculitis, hair loss, and oily hair. These problems can arise due to various factors, including poor daily habits, imbalanced nutrition, high stress levels, and exposure to toxic substances in the environment. Hair loss, also known as alopecia, is a common issue, and over one-third of women experience clinically significant hair loss at some point in their lives. The World Health Organization (WHO) has reported that approximately 70% of adults face scalp hair problems. These issues can stem from endocrine disorders, genetic factors, underlying diseases, or other internal causes. Research conducted in France has identified dandruff, scalp itching, and related symptoms prevalent among the population. Similarly, studies in the United States and Australia focusing on children have shown that 18% of children have dandruff. Therefore, scalp hair problems can affect both adults and children. Consequently, it is crucial to effectively prevent and maintain scalp hair health. In recent years, specialized services like scalp hair physiotherapy have emerged to address the increasingly severe scalp problems.[1]

Dandruff is a common condition that affects nearly half of the adult population after puberty, regardless of ethnicity or gender. It often causes an unpleasant cosmetic issue and can be accompanied by itching. The presence of Malassezia spp., a type of yeast, on the scalp is a significant factor in the development of dandruff. These yeasts have the ability to coat certain skin cells, known as keratinocytes. There is strong evidence suggesting that the skin cells themselves, called keratinocytes, actively contribute to the immune reactions seen in dandruff. When the Malassezia yeasts excessively colonize the keratinocytes, they trigger the release of substances that promote inflammation. This may explain the underlying micro inflammation observed in dandruff.[2]

The belief that microorganisms, including Pityrosporum ovale (a type of yeast), play a role in causing dandruff has been around for a long time. A century ago, Malassezia first suggested the involvement of the yeast Pityrosporum, a view later supported by Sabouraud and widely accepted today. The strength of this idea stems from observed differences in the microflora (microbial population) of scalps affected by dandruff. However, many early studies in this area are not as relevant today due to changes in the classification of both microorganisms and scaling disorders.

A fungus called Malassezia, similar to yeast, thrives on the natural oils found on most adults' scalps. Other factors that can contribute to dandruff include dry skin, sensitivity to hair care products, contact dermatitis, and underlying skin conditions like psoriasis and eczema. Dandruff can cause embarrassment in public settings and may also lead to hair loss.[3]

Currently, there are two main approaches to treating dandruff: using chemical-based anti-dandruff products and herbal-based alternatives. The market offers a variety of products and scalp treatments specifically designed to combat dandruff. However, some of these products contain chemicals that may have certain side effects. The botanical ingredients found in plants possess biological properties that act against the agents responsible for dandruff. On the other hand, synthetic products can have adverse effects such as itching, irritation, hair loss, nausea, headache, and increased scaling. In the traditional Indian medicine system, various plants, including neem, reetha, hibiscus, alo vera, flaxseeds, curry leaves, and fenugreek seeds, have been used for treating dandruff.

In recent decades, there has been a significant rise in the use of herbal-based cosmetics and herbal Shampoos, which contain essential oils and plant extracts. Traditional medicine in India has deep-rooted cultural and religious beliefs, and the World
Health Organization is involved in establishing guidelines to evaluate the effectiveness of traditional medicine. Medicinal plants are abundant in antioxidants, which are known to treat various diseases. The antioxidant potential of these plants is evaluated at different levels. Hair care preparations, such as creams, dyes, pomades, powders, and tonics, incorporate ingredients derived from plants. Therefore, combining the knowledge from Ayurveda and herbal medicine with evidence-based research on these plants can enhance their effectiveness. Medicines derived from herbal resources are easily accessible, cost-effective, safe, efficient, and rarely cause side effects. The demand for natural herbal medicines is increasing due to their lack of side effects and natural properties.[4]

Shampoos, serums, oils, and masks are the most commonly used cosmetic products for cleansing Hair and scalp in our daily lives. A natural product typically refers to a detergent solution that Contains suitable additives for additional benefits such as hair conditioning, lubrication, and Medication. Nowadays, herbal hair products are popular among consumers due to the belief that These natural-origin products are safe and free from side effects.[12] It is challenging to create a herbal Mask using a single natural ingredient that is milder and safer compared to non-herbal masks. Therefore, we have formulated a pure herbal hair mask using commonly used plant Materials with traditional usage to address dandruff and hair concerns. The pericarp of Sapindus Mukorossi, commonly known as soapnut or reetha, the seed of Linum usitatissimum (flaxseed).[5], Trigonella foenum-graecum (fenugreek seed), and Aloe vera have been included in the Formula. Soapnut and flaxseed produce rich lather when mixed with water due to their high Saponin content, which also has positive effects on the skin and other organ systems. Aloe vera is Known for treating hair loss, soothing the scalp, conditioning hair, reducing dandruff, and Unblocking hair follicles that may be blocked by excess oil. Fenugreek seeds, rich in protein.[6].

II. METHODOLOGY

1. ISOLATION OF DANDRUFF CAUSING ORGANISMS

Scalp sample were taken from adult person of age ranging from 20 to 25 years with the due permission from them. The sample collection was done using a sterile swab and comb scraping approximately One inch area using blunt scalpel and transferred into sterile sabouraud dextrose broth & Nutrient Broth and incubated overnight with shaking. Enriched cultures were streaked on Sabouraud dextrose agar (SDA), nutrient agar and salt Mannitol agar containing chloramphenicol (0.05%) and incubated at 30º C for three days. The pure colonies were studied by observing colony morphology and further identified (Table 2). The methodology has been followed by Kindo et al. (2004).

2. COLLECTION OF HERBS

The herbs were procured from local market and fresh plants. The herbs like flaxseed, Amla, alovera, curry leaves, Neem, onion, reetha & shikakai Hibiscus, fenugreek, & Tulsi were used to treat dandruff and hair related issues.

Ingredients -

- Flaxseed: Flaxseed is packed with nutrients and has several hair and health benefits due to its makeup of protein omega-3 fatty acids, fiber, antioxidants, vitamin E, lignans, or bioactive compounds, vitamin B they provide nourishment and faster hair growth.
- Amla : Amla, known as Indian gooseberry, is an ample source of vitamin C. It is full of many health benefits. Powdered Amla is used as an essential of hair tonics, required to enhance hair growth and improve hair pigmentation.[12]. It gives power to the roots, takes care of the colour, and improves luster. Amla oil application to the roots of hair improves hair growth and color.[9]
- Reetha : Reetha shows cooling effects and acts as an excellent cleanser on the skin. Soapnuts prevent the scalp from drying and retain the softness of the skin.[12]
- Neem : Neem helps to clean the scalp. It clears the clogged pores and improves hair growth. The regenerative properties are extremely essential for the treatment of dandruff. It is endowed with antiseptic and healing properties and it can be used for a variety of hair problems [11]. Dandruff can be removed by using neem leaves as a rinse. The most common neem uses are in our hair care rituals. As per Ayurveda, amla, reetha, neem, shikakai are herbs needed for healthy locks,enhanced hair growth, decrease hair fall and make the hair voluminous,[10].
- Hibiscus or ‘gudhal’: is the most beneficial ingredient for Hair[14]. It is used for the growth of hair, its regrowth, and hair Loss. Hibiscus carries amino acids, Vitamin A, C and alpha Hydroxyl acids along with other nutrients that are highly Beneficial for hair and scalp. They keep scalp healthy and Minimize the chances of dandruff from hair.[15]
- Onion : Onion juice is loaded with antioxidants that may help to prevent premature graying of the hair. Onion helps to nourish your hair and prevent hair fall. The antibacterial and antifungal properties of onion can help to prevent scalp infection and dandruff.[22]
- Curry leaves: Curry leaves are a natural hair tonic that can help to promote new hair growth and prevent hair loss. They are rich in nutrients such as beta-carotene, protein, iron, and vitamins B and C, which are essential for healthy hair growth[23].
- Aloe Vera : Aloe vera contains vitamins A, C, and E. All three of these vitamins contribute to cell turnover, promoting healthy cell growth and shiny hair.[25]

3. BIOCHEMICAL TEST

Biochemical tests are used to identify bacterial species by differentiating them on the basis of biochemical activities.[16] The difference in protein and fat metabolism, carbohydrate metabolism, enzyme production, compound utilization ability, etc. are some factors that aid in bacterial identification. Isolated Colonies were characterized on the basis of their morphology and cultural characteristics. After Morphological identification and biochemical confirmatory tests revealed name of isolates as Malassezia spp. And S.aureus.[24](Table 3).

4. FORMULATION OF HERBAL HAIR MASK

- To create the hair mask, various plant extracts such as curry leaves, neem, hibiscus, and Tulsi leaves are mixed in different proportions and dried for a few days before being ground into a fine powder
• Fresh amla (Indian gooseberry) is collected, cut into small pieces, crushed with water using a mortar and pestle, and filtered through a clean cotton cloth to obtain amla juice. Aloe Vera leaves were cleaned, drained of sap, and the pulp was collected, ground into a mixer to form liquid foam, and filtered to remove any particles.

• The liquid was then boiled at 70°C to form a uniform gel, eliminating any remaining yellow sap. Onions were collected, cut into small pieces, ground in a mixer, and filtered to obtain fresh onion juice. Fenugreek, shikakai, and reetha powder were obtained from the market and dissolved in distilled water.

• Flaxseeds were boiled in distilled water to obtain flaxseed gel, which was then filtered. The Herbal extracts and other ingredients were mixed in different proportions as given in Table 1 and boiled again to ensure thorough blending. Sterilized and airtight glass containers are used for storage.

5. EVALUATION AND ANTIMICROBIAL ACTIVITY OF FORMULATED HERBAL MASK

The herbal extracts were studied for antimicrobial activity against 5 isolates by agar cup diffusion Method [8].(Table 4). And after the formulation of the anti-dandruff hair pack, its anti-dandruff and antimicrobial activity checked by using Muller Hinton agar plate by well diffusion method. [11]. The antimicrobial activity of hair mask against Malassezia spp and Staphylococcus is shown in Table 5 and Fig. 1. The herbal mask was effective against all the isolates.

6. PHYTOCHEMICAL AND STABILITY ANALYSIS OF FORMULATED HERBAL HAIR MASK

The formulated product was studied for its phytochemical content w.r.t. carbohydrate, protein and saponin content. The stability testing of formulated hair mask was done by organoleptic methods. By utilizing sensory organs like eyes or nose, the organoleptic examination of the formulation was performed. The macroscopic characteristics of the product was done w.r.t. colour, odour and appearance. The stability in dark brown colour, distinctive herbal smell and smooth semisolid texture was noted.(Table 6).

Test for phytochemical content (Table No.7).
To Identify the phytoconstituents present in the products and their effect is shown on the body. Every plant exhibits certain phytochemical properties, which show a number of beneficial effects[18].

• Molisch Test : Molisch test is a colourimetric method for the analysis of the presence of carbohydrates in a given Analyte. This test is named after Austrian botanist Hans Molest. Molisch’s test is done by using Molisch reagent[9]

• Biuret Test : The biuret test is a chemical test that can be used to see if an analyte has peptide bonds or not. As a result, the biuret test may be used to figure out how much protein is in the analyte[10].

• Foam Test : The Foam test measures a lubricant’s foaming tendency and stability. [16]

<table>
<thead>
<tr>
<th>Name of the herb</th>
<th>Biological Name</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flaxseed</td>
<td>Linum usitatissimum</td>
<td>25gm</td>
</tr>
<tr>
<td>Amla</td>
<td>Phyllanthus Emblica</td>
<td>20ml</td>
</tr>
<tr>
<td>Alovera</td>
<td>Aloe barbadensis miller</td>
<td>20ml</td>
</tr>
<tr>
<td>Neem</td>
<td>Azadirachta indica</td>
<td>5ml</td>
</tr>
<tr>
<td>Reetha</td>
<td>Sapindus mukorossi</td>
<td>10gm</td>
</tr>
<tr>
<td>Shikakai</td>
<td>Acacia concinna</td>
<td>10gm</td>
</tr>
<tr>
<td>Hibiscus</td>
<td>Hibiscus rosa-Sinensis</td>
<td>25gm</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>Trigonella foenum-graeCU</td>
<td>10gm</td>
</tr>
<tr>
<td>Tulsi</td>
<td>Ocimum sanctum</td>
<td>10gm</td>
</tr>
<tr>
<td>Curryleaves</td>
<td>Murraya koenigi</td>
<td>10 ml</td>
</tr>
<tr>
<td>Onion</td>
<td>Allium cepa</td>
<td>10 ml</td>
</tr>
</tbody>
</table>

III. REDULT AND DISCUSSION
Total of 10 scalp samples were collected from the scalp of the individuals with dandruff. The age Group of the participants was in the range of 18-25 years using sterile cotton swab were isolated On Sabouraud’s agar and salt mannitol agar at 30°C and incubated for 2 to 3 days at37o C. All the Plates were observed for morphologically distinct colonies All the distinct colonies were then studied for their morphological, cultural and biochemical characteristics (Table 2 and 3).

<table>
<thead>
<tr>
<th>Characterization</th>
<th>Colony 1</th>
<th>Colony 2</th>
<th>Colony 3</th>
<th>Colony 4</th>
<th>Colony 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>Cream to yellow</td>
<td>Yellow</td>
<td>Yellow</td>
<td>White</td>
<td>Creamy white</td>
</tr>
<tr>
<td>Size</td>
<td>7mm</td>
<td>5mm</td>
<td>3mm</td>
<td>2mm</td>
<td>4mm</td>
</tr>
<tr>
<td>Shape</td>
<td>Slightly wrinkled</td>
<td>Circular</td>
<td>Oval</td>
<td>Circular</td>
<td>Oval</td>
</tr>
<tr>
<td>-------------</td>
<td>------------------</td>
<td>----------</td>
<td>------</td>
<td>----------</td>
<td>------</td>
</tr>
<tr>
<td>Consistency</td>
<td>Soft</td>
<td>Smooth</td>
<td>Dry</td>
<td>Creamy</td>
<td>Mucoid</td>
</tr>
<tr>
<td>Opacity</td>
<td>Translucent</td>
<td>Opaque</td>
<td>Translucent</td>
<td>Opaque</td>
<td>Opaque</td>
</tr>
<tr>
<td>Margin</td>
<td>Irregular</td>
<td>Entire</td>
<td>Irregular</td>
<td>Entire</td>
<td>Irregular</td>
</tr>
<tr>
<td>Gram staining</td>
<td>Positive ovoid shaped cell</td>
<td>Positive cocci</td>
<td>Negative cocci</td>
<td>Negative rods</td>
<td>Negative rods</td>
</tr>
</tbody>
</table>

Table No. 3 BIOCHEMICAL CHARACTERIZATION OF THE ISOLATES

<table>
<thead>
<tr>
<th>Biochemical Test</th>
<th>Isolate 1</th>
<th>Isolate 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Mannitol</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Lactose</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Nitrate</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Indole</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Citrate</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Starch</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>VP</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>BPA</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Oxidase</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Catalase</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>Gram staining</td>
<td>Gram positive cocci</td>
<td>Gram positive cocci</td>
</tr>
<tr>
<td>Identified as</td>
<td>Malassezia spp</td>
<td>Staphylococcus aureus</td>
</tr>
</tbody>
</table>

Table No. 4 : ANTIMICROBIAL ACTIVITY OF HERBAL EXTRACT AGAINST STAPHYLOCOCCUS and MALASSEZIA ISOLATES

<table>
<thead>
<tr>
<th>Extracts/isolates</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flaxseed</td>
<td>12mm</td>
</tr>
<tr>
<td>Curry Leaves</td>
<td>13mm</td>
</tr>
<tr>
<td>Hibiscus</td>
<td>16mm</td>
</tr>
<tr>
<td>Amla</td>
<td>18mm</td>
</tr>
<tr>
<td>Neem</td>
<td>-</td>
</tr>
<tr>
<td>Fenugreek</td>
<td>14mm</td>
</tr>
<tr>
<td>Shikakai</td>
<td>15mm</td>
</tr>
<tr>
<td>Reetha</td>
<td>12mm</td>
</tr>
<tr>
<td>Onion</td>
<td>-</td>
</tr>
<tr>
<td>Malassezia spp</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12mm</td>
</tr>
<tr>
<td></td>
<td>13mm</td>
</tr>
<tr>
<td></td>
<td>16mm</td>
</tr>
<tr>
<td></td>
<td>18mm</td>
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<td></td>
<td>-</td>
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<tr>
<td></td>
<td>14mm</td>
</tr>
<tr>
<td></td>
<td>15mm</td>
</tr>
<tr>
<td></td>
<td>12mm</td>
</tr>
<tr>
<td>Malassezia spp</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>12mm</td>
</tr>
<tr>
<td></td>
<td>12mm</td>
</tr>
<tr>
<td></td>
<td>17mm</td>
</tr>
<tr>
<td></td>
<td>19mm</td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>15mm</td>
</tr>
<tr>
<td></td>
<td>14mm</td>
</tr>
<tr>
<td></td>
<td>13mm</td>
</tr>
<tr>
<td>Malassezia spp</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10mm</td>
</tr>
<tr>
<td></td>
<td>12mm</td>
</tr>
<tr>
<td></td>
<td>15mm</td>
</tr>
<tr>
<td></td>
<td>12mm</td>
</tr>
<tr>
<td></td>
<td>9mm</td>
</tr>
<tr>
<td></td>
<td>13mm</td>
</tr>
<tr>
<td></td>
<td>12mm</td>
</tr>
<tr>
<td></td>
<td>9mm</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>10mm</td>
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<tr>
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<td>10mm</td>
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<td>-</td>
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<tr>
<td></td>
<td>-</td>
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<tr>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus spp</td>
<td>-</td>
</tr>
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<td></td>
<td>-</td>
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<td></td>
<td>10mm</td>
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<td>12mm</td>
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<td>12mm</td>
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<td></td>
<td>10mm</td>
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<tr>
<td></td>
<td>10mm</td>
</tr>
<tr>
<td></td>
<td>11mm</td>
</tr>
<tr>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>
Table No. 5: Antimicrobial activity of hair mask against *Malassezia* spp and *Staphylococcus* spp

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Zone of inhibition in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Malassezia 1 furfur</em></td>
<td>20 mm</td>
</tr>
<tr>
<td><em>Malassezia 2</em></td>
<td>12mm</td>
</tr>
<tr>
<td><em>Malassezia 3</em></td>
<td>-</td>
</tr>
<tr>
<td><em>Malassezia 4</em></td>
<td>10mm</td>
</tr>
<tr>
<td><em>Malassezia 5</em></td>
<td>11mm</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>18 mm</td>
</tr>
</tbody>
</table>

Fig: Antimicrobial activity of hair mask against *Malassezia* spp and *Staphylococcus* spp

Stability and Shelf-life analysis of formulated herbal hair mask

Various tests were performed, as mentioned in Table 7, to identify the phytoconstituents present in the products. Every plant exhibits certain phytochemical properties, which show a number of beneficial effects.

The safety of the formulation was checked by Patch test by applying, the tiny amount of Formulation on the surface of the hand and the observed for redness, irritancy and itchiness and Swelling caused by formulation after 5 minutes (Table 8).

The hair mask was held on for one month at 30 to 40° C to study its shelf life in sterile air tight Glass jar. Organoleptic evaluation signified that the pack is characteristic smelled, coarsely Powdered. Physicochemical parameters revealed that the loss on drying was as minimal. pH was Found to be neutral. Fit the requirements of different hair types. It confirmed the presence of main Phytoconstituents, which provide full nourishment for the scalp as well as hair. Irritancy test was Found to be negative for redness and swelling as the contents were used in their natural form, Devoid of artificial additives. Its moisture content was also found to be very less. Stability tests Performed signified the compatible nature of the formulation, for the observation of colour, odor, Appearance, texture, and pH. The formulation is made with herbal ingredients, signifying few Chances of the decomposition of the formulation, as there is a humid substance in either raw or finished form

The formulation was kept under observation for one month at room temperature to Look for any visible changes in its color, odor, texture and appearance. The formulation was kept Under observation for one month at room temperature to look for any visible changes in its color, Odor, texture and appearance. The pH was also noticed. The formulation can be stored easily and Is suitable to be used at any temperature, at any place in its stable form, since it is a natural herbal Based formulation, with no harmful chemicals. However, the frequent use of it provides Voluminous, smooth and dandruff free hair.
Table No 6 : Stability Testing of formulated hair mask by organoleptic methods

<table>
<thead>
<tr>
<th>Organoleptic property</th>
<th>Observation (0Day)</th>
<th>Observation (30 Day) at 30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Dark brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Odour</td>
<td>Herbal</td>
<td>Herbal</td>
</tr>
<tr>
<td>Texture</td>
<td>Smooth and semi-solid</td>
<td>Smooth and semi-solid</td>
</tr>
<tr>
<td>pH</td>
<td>7.5</td>
<td>8.0</td>
</tr>
</tbody>
</table>

Table No 7 : Phytochemical Evaluation of formulated herbal hair mask

<table>
<thead>
<tr>
<th>Test</th>
<th>Purpose for detection</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molish test</td>
<td>Presence of carbohydrates</td>
<td>Positive</td>
</tr>
<tr>
<td>Biuret test</td>
<td>Presence of proteins</td>
<td>Negative</td>
</tr>
<tr>
<td>Foam test</td>
<td>Presence of saponin</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Table No 8 : Evaluation of application safety of the formulated hair mask by patch test

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Effects of formulation on skin</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swelling</td>
<td>No swelling</td>
<td>No swelling</td>
</tr>
<tr>
<td>Redness</td>
<td>No red colour</td>
<td>No red colour</td>
</tr>
<tr>
<td>Irritation</td>
<td>No irritation</td>
<td>No irritation</td>
</tr>
</tbody>
</table>

Application instructions of Anti-dandruff Hair Care Formulation:
The pack should be applied on a weekly basis on hair, semi-solid paste can be applied directly. It should be spread evenly on the hair with the help of a brush, from roots to the hair tip. It should be left for 30 minutes. Then it should be removed by gently washing with plain water. Such application instruction ensures maximum benefit of anti-Dandruff Hair mask along with hair nourishment, preventing graying of hair, reduction in hair loss and shine and luster to the hair.

IV. CONCLUSION
Hair is one of the external barometers of internal body conditions. It is an important part of human body. Hair loss is a major problem among youngsters as well as the adults. It is a serious distressing condition psychologically as well as socially and cosmetically. The prevalence of hair fall in India is about 60%, dandruff is 20%, and baldness is about 50%. According to Ayurveda, the condition of gradual loss of hair is called Khaliya. There are many reasons for hair loss which may even vary from the nutritional disorders to severe drug reactions. One of the major causes for hair loss is dandruff. In this study total 10 scalp sample were collected from individuals with dandruff from the age group of 18 to 25 years and isolation was done on Sabouraud’s agar and mannitol salt agar. Isolated Colonies were characterized on the basis of their morphology and cultural characteristics. After Morphological identification and biochemical confirmatory tests revealed name of isolates as Malassezia spp. And S. aureus. The herbs were collected from local market and herbal hair mask was formulated using herbal extracts. The formulating the herbal hair mask was studied for its antidandruff and antimicrobial activity using Müller Hinton agar plate by agar well diffusion method. Both the isolates, Malassezia spp and Staphylococcus aureus found to be inhibited and clear zone of inhibition was observed around the well containing hair mask. The stability and patch test were done to checked side effects of the herbal mask and no Irritation, redness, itchiness were observed. Novelty of Formulation includes as the formulation is in convenient form of a pack, no artificial additive has been incorporated, the constituents are easily accessible, so they can be prepared easily at home and can be made freshly before use. The study thus concludes the successful formulation of herbal hair mask made with 100 % natural and pure herbal ingredients without any side effects and antidandruff activity. The herbal formulation is totally safe, effective and affordable and hold potential to offer overall hair health of human.

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