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Preparation and Characterization of Polyherbal Hair Cream for Stimulating the Hair Growth Activity

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ABSTRACT
Natural remedies are more acceptable today because they are safer and have fewer sideeffects than synthetic ones. The demand for herbal products on the world market is growing. Herbs are mainly used to show therapeutic effects through cosmetic formulation and are also used to make the beautification of the body. This article dealswith the preparation and evaluation of a multi-herbal hair growth cream using the leaves, flowers, and fruits of the plant. Although there are different formulations available on the market, we use three different types of extracts to fight effective anti- inflammatory, anti-bacterial, and anti-fungal agents. We recommend using amla extract, which has good antifungal and antioxidant properties, and hibiscus extract, which has effective antibacterial and antioxidant properties, and Brahmi extract, which has effective anti-inflammatory and antioxidant properties. Different batches of the formulation namely F1, F2, and F3 with different concentrations were prepared. The prepared formulations (F1 to F3) were evaluated according to several parameters suchas appearance, pH, viscosity, dye test, solubility test, irritation test, acid content, saponification value, homogeneity, spreadability, etc. Formulation F2 had good dispersibility, good consistency, homogeneity, appearance, pH, easy removal and no signs of phase separation. Formula F2 shows no redness, inflammation, swelling or irritation during the irritation test. These preparations are safe to use on the hair and scalp.
Keyword: *Brahmi, Hibiscus, Amla and Herbal Formulation, Herbal Hair Cream.*

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INTRODUCTION

Herbal cosmetics

Natural cosmetic and herbal cosmetic are similar terms. Ancient humans and their cultures were already aware of beauty and cosmetics. People now prefer the best because they are more health conscious than ever before. The term "Herbal Cosmetic" refers to products that have been specially designed with one or more herbal substance to solely provide specific cosmetic benefits. These products are made with a base of different cosmetic ingredients that are legal to use. The popularity of herbal cosmetic is rising nowadays, not only in industrialised nation like the United States, Canada, the United Kingdom, Australia, Germany, and France, but also in less developed nation like the Philippines, China, and India, among others. Herbs don't provide rapid relief. They provide a means of bringing the body into proper harmony with nature. Due to their skin friendliness and absence of adverse effects, herbal treatments are becoming more and more popular. The wonderful thing about herbal cosmetic is that they are completely created from herbs and shrubs and have no adverse effects on the human body and instant give it nutrition and other beneficial minerals [1, 2]. Although hair is simple in structure, it plays an important role in social functions. The human body needs hair to function properly. It can cause a variety of issues, such as thinning hair, dullness, dandruff, unmanageable hair, lack of volume, conditioning, immature greying and hair loss. The shape, length, diameter, texture, and color of the hair can all very. The hair's cross section may also be round. Whetherit is triangular, crooked, or flattened, it affects how the hair curls. There is hair on all mammals. Its primary goal is to maintain body temperature. It also aims to operate asa way organ, reduce friction, and protect against sunlight. A person's hair is their greatest asset, and it has a significant impact on their quality of life. In the past hairs were thought of as a protective covering for the scalp. No matter to whatever gender a person belongs, having hair can help them feel confidant and proud of themselves. One's

desire to have black, healthy, lustrous, and high-quality hairs has always been their greatest aspiration. No matter how long or how short they are, maintaining them is a top responsibility for everyone. [3]
Mycotic condition (Fungal Infection): A fungal skin illness known as a scalp yeast infection is brought on by a yeast called candida. On your scalp, it results in an itchy, flaky rash. Warm, dark surroundings are ideal for Candida growth. Malassezia speciesare known to produce both seborrheic dermatitis and dandruff, which are two symptoms of the same disease. [2]
Parasitic capitis (Bacterial Infection): Pediculosis capitis is a ubiquitous parasitic skin disease caused by pediculus humanus capitis. The only places where head lice can reproduce are on the scalp and hair of human. Transmission occurs by direct head-to-head contact. Important bacterial pathogens are carried by head lice.
Inflammatory condition: The body's immune system will interpret the body's cells and organs as foreign organisms and fight them. The body's immune system frequently destroys the hair follicles because it perceives them as foreign invaders. Alopecia, or inflammation-based hair loss, is the result of this. The distinctive feature of psoriasis is discrete erythematous plaques covered in silver-gray scale.[2]
Androgenetic alopecia (AGA) is the most common from of hair loss. When it affects women, it causes diffuse alopecia (female pattern hair loss) over the mid-frontal scalp. This process is a result of hair follicle miniaturization withinunits. The diameter, colour, and length of the hair shaft all gradually decrease. AGA is distinguished by these little hairs. Most women AGA have normal mensesand pregnancies. Androgens cause this condition in people with the genetic predisposition to it. These patients present hair follicle with increased 5α- reductase activity and di-hydro-testosterone (DHT) levels. In these genetically susceptible hair follicles, the DHT Binds to the androgen receptor and the hormone-receptor complex, activating the genes responsible for the transmission of the normal hair follicle in miniaturized follicles. A generalised alopecia will result from the decrease in terminal fibres per follicular unit. Androgenetic alopecia mostly has a psychological effect. While men anticipate hair loss associated with ageing, it typically comes as a surprise and is unwelcome at any time in women [4-8].
Alopecia areata (AA) is an autoimmune, inflammatory disorder that causeshair loss on the scalp and/or body. It affects up to 2% of the population and it is characterized by patchy hair loss. Alopecia universalis, which results in the loss ofall body hair, can also affect the entire scalp. According to histopathology, the peribulbar area has an elevated number of catagen and telogen follicles as well as an inflammatory lymphocytic infiltration [9].
To better understand the pathophysiology of AA, many factors are being studied. Genetic makeup, autoimmune reactions with organ-specificity, and non- specific immune responses are potential reasons [10].

MATERIAL AND METHODS

Collection of plant materials: Crude plant powdered material was purchased from Dhanvantari Aushadhalaya and check basic quality control parameters then powdered material was subjective to extraction process by Soxhlet apparatus.

Fig 1: Powder of *Bacopa monnieri, Hibiscus Rosasinesis, Phyllanthus emblica*

Procedure:

The finely powdered material undergoes the extraction method by using hydroalcoholic solvent (70% ethanol and 30% water) for a duration of three successive hours the temperature should not exceedingly more than 100°C. The dark green color hydroalcoholic extract was collected, filtered and the filtrate was use for further study. The concentrated product was stored in the refrigerator to preserve it, and it was then utilized in additional experimental trials. The same procedure was followed for the crud drug Brahmi, hibiscus, amla.
Soxhlet Extraction / Solvent Extraction

- A dried powdered of 7.5 g of the Brahmi powder was weighed, and the weight recorded. (5g , 7.5g , 10g weight of powder)
- The extraction was carried out using ethanol as the extraction solvent. In the Soxhlet apparatus, the solvent in the round bottom flak was heated from the heating mantle to become evaporated and got condensed down through the sample where it was able to extract the chemical constitution.
- The same procedure of Soxhlet Extraction was followed for the crud drug brahmi, hibiscus, amla.

Procedure of formulation: -

Oil in water (O/W) type herbal hair cream was finalize for the study. The emulsifier (stearic acid) and the other oil soluble component (bees wax, lanolin, ceto stearyl alcohol, *Olea europea, Ricinus communis*) were dissolved in the oil phase (part A as oil phase). The other water-soluble components (Extract of *Bacopa monnieri, Hbiscus rosa sinensis, Phyllanthus emblica*) and triethanolamine were dissolve in the aqueous phase (part B as a water phase). Both the mixture A and B heated on the water bath until reaches 70^0 C. After that aqueous phase was mixed with the oil phase with constant stirring. Finally add preservatives and perfume. The formula for the cream is provides in table-1.

Table: Composition of cream % w/w

INGRENDIENTS	F1 (5%)	F2 (7.5%)	F3 (10%)
Bacopa monnieri extract	1	1	1
Hibiscus Rosa sinesis extract	1	1	1
Phyllanthus emblica extract	1	1	1
Stearic acid	5	5	5
White bees wax	2	2	2
Ceto stearyl alcohol	4	4	4
Lanoline	1	1	1
Castor oil	1	1	1
Olive oil	4	4	4
Methyl paraben	0.02	0.002	0.002
Triethanolamine	2	2	2
Water	Upto 100	Upto 100	Upto 100
Perfume	Qs	Qs	Qs

EVALUATION PARAMETER

Appearance: The appearance of the cream was found by observing its color, pearlescence, roughness, and graded. [11]

pH of the cream: The pH meter was calibrated using the standard buffer solution and measured the pH. About 0.5g of the cream was weighed and dissolved in 50.00ml of distilled water and its pH was measured and recorded. [12]

Viscosity: A Brookfield Viscometer was used to calculate the viscosity. Brookfield Viscometer (LMDV-60) using spindle number SPL-4 at a 12 rpm at a temperature of 25^0C. Three readings were taken, and the average of those three was recorded for the determination.[12, 13]

Dilution test: The emulsion is diluted in this test either with water or oil. The emulsions are the diluted with the water, it will remain stable as water is the dispersion medium, thisemulsion is O/W type emulsion. However, if it is diluted with oil, the emulsion is unstable and will break because water and oil do not mix. While water in oil emulsioncan be diluted with an oily liquid, oil in water emulsion can be easily diluted with an aqueous solvent. [14]

Dye solubility test: Soluble dyes make the color according to their phases of solubility. While oil soluble dyes make the color according to the oil phase and water soluble dye make thecolor according to water phase. Either a water-soluble dye such as amaranth or an oil-soluble dye such as scarlet red dye or sudan III are mixed with the cream. Place a small quantity of the cream on a microscopic slide cover it with a cover slip, and examine it under the microscope. In the case with oil-soluble dye, in this test disperse globules appear red color and the background colorless that is O/W type emulsion. In the case with water- soluble dye, in this test disperse globules appear colorless are visible on a colorless background, that is W/O type emulsion.[13, 14]

Acid value: Prepare the solution of 0.1N sodium hydroxide (NaOH) and phenolphthalein indicator. 2gm of phenolphthalein powder are dissolved into 100ml of ethanol. 0.1N NaOH solution prepare as 4gm of sodium hydroxide are dissolved into 1000ml of distilled water. Prepare the sample solution. Take the 10gm of cream into conical flask and keep it a side. Take 50ml of ethanol (99%) into 150ml of conical flask

and add 2-3 drops of phenolphthalein indicator and shake, than add 0.1N NaOH drop wise until the light pink color appears. Light pink color appears means the solution are neutralized the ethanol, this ethanol are added into sample and mix the solution. Heat the solution on hot plate until it dissolves completely.[14] Record the reading on burette and add few drops of phenolphthalein indicator into sample than titrate with 0.1N NaOH and continues stirring until pink color appears.

$$\text{Acid value} = MW_{NaOH} \times N \times V / WS$$

Where,

WS = Sample weight

N = Normality of NaOH

MW_{NaOH} = Molecular weight of NaOH

V = Volume of NaOH solution is final reading – initial reading

Saponification value:

Take the 2g of cream refluxed with 25ml of 0.5N alcoholic KOH for 30 minutes, make sure the sample should be dissolve completely. Then after that add phenolphthalein indicator for appear the light pink color and along with that prepare the blank solution for further calculation. After add the indicator titrated immediately, with 05N HCL ('a'ml). The blank was carried out similarly ('b' ml) without the sample. [14]

$$\text{Saponification value} = 28.05(b-a)/W$$

Where,

a = The volume in ml of titrant (with sample)

b = The volume in ml of titrant (without sample)

w = The sample's weight in grammes

Homogeneity: By touch and visual inspection, the homogeneity of the formulation was evaluated. [15]

Irritancy test: The small quantity of cream was applied on left hand of dorsal side surface of 1sq.cm and time was noted. Irritancy, edema, erythema was observed in if any for regular interval up to 24hr and reported. [14]

Accelerated stability studies: On all of the formulations, accelerated stability tested were carried out by keeping them at room temperature for 20 days at regular intervals. Homogeneity, viscosity, physical changes, pH, and the type of smear were among the variables examined during the stability investigations.[14]

Smear Type: When herbal cream was applied to the skin for the test, either an oily or waterysmear was created. [15]

Removal: The cream that had been applied to the skin was removed by washing under running water and exerting as little effort as possible. [15]

ANTIMICROBIAL ACTIVITY OF HERBAL HAIR CREAM

Bacterial organisms test: In the investigation, staphylococcus aureus was used as test organisms. The microorganisms that had been morphologically recognised were them put through a biochemical test to determine their identify up to the biochemical level. [14]

Antibacterial Assay

- **Preparation of inoculums**

On slopes of nutrients agar, stock cultures were kept at 4^0C. The experiment's active cultures were made by transferring a loopful of cells from the stock cultures into a test tube filled with Muller-Hinton broth (MHB) for bacteria, which was incubated for 24 hours at 37^0C without being stirred. Fresh Muller-Hinton broth was used to dilute the cultures until they reached optical densities that corresponded to 2.0×10^6 colony forming units (CFU/ml) for bacteria.

- **Preparation of sterile swabs**

To prepare and sterilize cotton wool swabs on wooden applicators or plastics, or exclusively for the wooden swabs, dry heat was used. By placing the swabs in culture tubes, papers, tins, etc., it was sterilized.

- **Sterilization of forceps**

By dipping forceps in alcohols and then burning off the alcohol, forceps can be sterilized.

- **Preparation of Muller-Hinton agar**

One litter of distilled water was combined with 38 milligrams of Muller- Hinton agar powder in a flat—bottomed conical flask. In order to completely dissolve the media, the liquid was heated while being stirred frequently. After using cotton wool to seal the flask tightly and further covered with aluminium foil. The mixture was autoclaved for 15 minutes at 121^0C before being allowed to cool to room temperature. In order to achieve a uniform depth of 3-4 millimeters, the media was poured into the petri dishes in a laminar flow. The media-filled petri dishes were then put in a sterile plastic bag and kept at a temperature of 2-8

degrees Celsius until they were needed.

Table: chemical evaluations of herbs used in formulation for the phyto-constituents.

Name of analysis	*Phyllanthus emblica (amla)*	*Hibiscus rosa sinensis (hibiscus)*	*Bacopa monnieri (brahmi)*
Alkaloids	-	-	+
Carbohydrate	+	-	-
Proteins	-	+	-
Saponins	+	-	+
Glycosides	-	+	+
Vitamin C	+	+	-
Amino acid	-	+	+
Steroids	+	-	-
Flavonoids	+	+	+

RESULTS AND DISCUSSIONS

Formulations F1, F2, and F3 were successfully completed using their respective procedures, and after they had been tested for stability, the basic standardisation parameter had been determined and displayed in the table below.

Table: Results and Discussion

S.No.	Parameter	F1	F2	F3
1	Appearance	White	Off White	Light brown
2	Ph	6.1	5.3	6.4
3	Viscosity	27003-27085 cps	27008-27093 cps	27010-27098 cps
4	Dye test	O/W emulsion	O/W emulsion	O/W emulsion
5	Acid test	1.38	1.029	1.98
6	Saponification Value	20.03	26.71	40.07
7	Homogeneity	Uniform distribution and smooth	Uniform distribution and smooth	Uniform distribution and smooth
8	Irritancy test	No irritation	No irritation	No irritation
9	Stability test	No phase separation	No phase separation	No phase separation
10	Dilution test	O/W emulsion	O/W emulsion	O/W emulsion
11	Type of Smear	Non-greasy	Non-greasy	Non-greasy
12	Removal	Easily remove by water	Easily remove by water	Easily remove by water

Here Total of three formulations (F1, F2 and F3) were developed and assessedhere. F2 formulation is more stable than F1 and F3 formulation, according to the evaluation study. In the current project, it was determined to extract crude powder drug and create herbal hair cream. The herbal hair care had an O/W type emulsion, making it simple to remove with water and reducing customer complaints. Therefore, F2 waschosen as the better formulation for additional in-vitro research.

Fig.: Poly-Herbal Hair Growth Cream

Appearance: The prepared cream was found to be of a off white color and has pleasant odor.
Ph: The pH of the cream was found to be 5.3, which is acidic value.
Viscosity: The viscosity of cream was found to be 27008 cps.
Dye test: The cream was found to be of the O/W type emulsion by Dye test
Acid value: The acid value of the cream was found to be 1.029.
Saponification value: The saponification value of the cream was found to be 26.71
Homogeneity: It was found that the cream had a homogenous, smooth, and constant silkytexture.
Emolliency: Following observation, it was discovered that the cream did not leave anyresidue on the scalp's surface or the skin after use.
Dilution test: The cream was found to be of the O/W type emulsion by Dilution test.
Type of smear: It was found that the cream produced non-greasy film on the scalp and skinsurface.
Removal: It was found that the cream was removed by water easily on the scalp andskin surface.
Antimicrobial Activity of Herbal Hair Cream:-The Poly-herbal hair growth cream (F2) was evaluated for *in-vitro* antimicrobial activity here the stains were observed for this study, that is *staphylococcus aureus* (bacterial). This study revealed that Poly-herbal hair growth cream exhibited significant antimicrobial activity.
The three formulations (F1, F2 and F3) were developed and assessed here. F2 formulation is more stable than F1 and F3 formulation, according to the evaluation study. In the current project, it was determined to extract crude powder drug and create herbal hair cream. The herbal hair care had an O/W type emulsion, making it simple to remove with water and reducing customer complaints. Therefore, F2 was chosen as the better formulation for additional in-vitro research.

CONCLUSION

This study concluded that, the combining extract of *Bacopa monnieri, Hibiscus rosa sinensis, Phyllanthus emblica* were used in different concentration to get hair growth effects on scalp and hair such as antimicrobial, prevent hair fall, increased hair growth and follicles, prevent greying of hair, prevent dryness of hair and scalp. We are aware that a single plant cannot produce an effective effect, but by combining many extracts, a formulation's efficacy can be increased. Based on the results, F2 formulation was more stable than F1 and F3 formulation, it was confirmed by evaluation study. So, the further *in-vitro* studies were completed with F2 formulation and its shows significant antimicrobial activity.

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