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Formulation and Evaluation of Herbal Hair Serum and a Comparative Study with Marketed Formulations

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ABSTRACT: The main objective of the study is to formulate and evaluate the herbal hair serum and determine the physicochemical function that emphasis on safety, efficacy and quality of the product. A hair serum is a light weight, liquid-based hair product commonly used to add shine, control frizz, and protect against heat damage by creating a protective layer. Addition of natural hair growth stimulants like flax seed, fenugreek and castor oil make the formulation better for hair growth. So it can be applied on hair as well as scalp. The ingredients such as flax seed, fenugreek, castor oil and vitamin E deals with hair growth by strengthening and nourishing the hair follicles. Other ingredients used in the preparation are Aloe vera, Orange peel and Reetha. Aloe vera and Orange peel act as antioxidant and Reetha provides surfactant action. As the majorities of the existing hair serums are synthetic in origin and have several side effects, the present study was performed to formulate hair serum containing various natural ingredients. This will helps to avoid side effects and promote hair health. Various studies and tests are performed on the ingredients and four formulations were made. All these formulations were subjected to evaluation of its organoleptic properties, pH, skin irritation test, fragrance, and spread ability and stability studies. The stability studies indicated that prepared hair serum was stable throughout the studies. By comparing the evaluation test results of four formulations, the project aims to spot appropriate one best formulation. The selected best formulation of herbal hair serum was compared with two herbal hair serums available in the market. The results were found to be satisfactory. The findings of the study will contribute to the development of herbal hair serum which promote hair growth and with minimal side effects.

KEY WORDS:flax seed, fenugreek, antioxidant surfactant, surfactant, aloe vera, reetha

I INTRODUCTION

Hair is a crucial aspect of human appearance and place a significant role in enhancing beauty. It is characterized as an improved epithelial structure formed through the keratinization of germinated cells, emerging from follicle in the skin. Hair is present on various parts of the body including the scalp, skin and face, making proper care essential for maintaining its health and aesthetic appeal [1].

Human hair Human hair is primarily composed of 65-95% proteins, predominantly keratin, which accounts for about 80% of its structure. Keratin is a sulfur-rich protein that contributes to hair's strength, flexibility, and durability through its complex structure. Hair's physicochemical properties and shape are determined by the organization of its proteins. The type of hair produced is influenced by the size and shape of hair follicles; larger follicles yield terminal hairs (such as those on the scalp), while smaller follicles produce fine body hair (vellus). Curved follicles are responsible for curly hair across different ethnicities. A hair consists of a hair shaft (visible part sticking out of the skin) and a hair root (in the skin, extending down to deeper layers). Each hair root is located in a hair follicle, surrounded by a tiny muscle (arrectorpili) that makes hairs stand up and is connected to a sebaceous gland. At the base of the hair, there's a round hair bulb with a hair papilla that supplies the hair root with blood and allows new hair cells to form. New cells constantly form in the hair bulb, stick together, and harden, gradually pushing a single hair out of the skin at a rate of 1 cm per

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month [2]. Hair consists of several layers and components that contribute to its characteristics:

Hair serum Serums are concentrated topical products designed for skin and hair care, containing small molecules of active constituents. They have a thin consistency and deliver targeted benefits. While hair serums primarily focus on the hair, scalp serums target the scalp; however, many formulations are versatile enough to be used on both hair and scalp[3]. Hair serums gained popularity in the 1980s and 1990s as a new category of hair care products. Formulated with lightweight oils, silicones, and conditioning ingredients, these serums were designed to reduce frizz, enhance shine, and protect hair from environmental factors. Initially used primarily in professional salons, they eventually became accessible for home use, allowing consumers to benefit from their properties [4].

Natural hair serum

Herbal hair serum is a liquid, leave-in hair treatment formulated with natural plant-based ingredients, such as herbal extracts, essential oils and botanical nutrients. It is designed to nourish the scalp, strengthen hair, reduce frizz, add shine and promote healthy hair growth without the use of harsh chemicals. Herbal hair serum are often used to protect hair from damage, prevent split ends, and improve overall hair texture[5].

Benefits of herbal hair serum

•Natural ingredients: Herbal hair serum are made from natural ingredients, reducing the risk of chemical damage

• No harsh chemicals: Herbal hair serums are free from silicones, parabens, sulfates and other harmful additives, making them safer for long term use.

• Scalp nourishment: Herbal serums often include ingredients like aloe vera, amla and neem, which promotes scalp health, reduce dandruff, and improve hair growth.

•Reduced hair fall: The natural ingredients strengthen hair follicles and minimize hair fall, unlike synthetic serums that mainly focus on appearance rather than hair health.

• Improves hair texture naturally: Herbal serums smooth frizz, add shine, and make hair more manageable without excessive reliance on artificial silicones.

• Gentle on sensitive scalps: Ideal for people with sensitive scalps or allergies, as they are less likely to cause irritation or allergic reactions.

• Encourages hair growth: Ingredients like castor oil, fenugreek and hibiscus stimulate hair follicles, promoting thicker and healthier hair growth.

•Eco-friendly: Herbal serums are often biodegradable and eco-friendly, reducing environmental impact compared to chemical based serums [3][4],[5],[6].

II. MATERIALS AND METHODS

The ingredients used were of natural origin and their properties are.

1. Flaxseed: Anti-inflammatory, Anti-oxidant, Hair growth stimulant
2. Aloe vera: Anti-inflammatory,Anti-oxidant, Conditioning agent
3. Fenugreek: Hair growth stimulant, Antibacterial
4. Orange peel: Anti-inflammatory, Anti-oxidant
5. Reetha: Surfactant,Anti-inflammatory and Fungicidal
6. Castor oil: Anti-inflammatory, Hair growth stimulant, Anti-dandruff, Provides nourishment to hair root
7. Vitamin E:Anti-oxidant agent, Prevent premature greying

Preparation of reetha extract:Reetha fruit was collected and dried in a micro-oven at 70^0C. Seeds from the dried fruit were separated and the pericarp was finely powdered. Weighed 5g of reetha powder and added the powder in a beaker containing 100ml of water (1:20). Heated the mixture gently 50^0-60^0C for 1-2 hours with continuous stirring. Filtered the mixture through muslin cloth[1].

Preparation of flaxseed extract: Weighed 5g of flaxseed and heated with 50ml distilled water for 15 minutes. Filtered the resulting solution using muslin cloth and allowed to cool[1].

Preparation of aloe veraextract:collected 2-3 fresh aloe vera leaves and kept for 15 minutes in water to remove toxins. Scrapped the gel from leaves using knife and collected the gel. The obtained gel was grinded. Filtered the resulting solution using muslin cloth [3].

Preparation of fenugreek extract: Weighed 2g of fenugreek.Transferred it into a beaker containing 20ml distilled water. Boiled the solution for 5 minutes and allowed to cool. Filtered the solution using filter paper[1].

Preparation of orange peel extract: The orange peel was collected, dried and powdered. 5g of orange peel powder was taken in beaker containing 40ml of water. Boiled for 5minutes and filtered. The solution was kept in refrigerator in a closed container[1].

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Preformulation studies of herbal extracts

Reetha

Loss on drying: Weighed the silica crucible. Transferred 1g of sample into the crucible. Taken the weight of sample and crucible. Placed the crucible with reetha in oven. Heated for 3 hours at 105^0C. Weighed in an interval of 1 hour until a constant weight is obtained [7].

pH: Weighed 5g of reetha extract and boiled it in 100ml of distilled water for 10-15 minutes. Allowed to cool the solution and filtered. pH of the sample is determined using pH meter

Ash Value: Weighed clean, dried empty silica crucible. Weighed 2g of the reetha extract using an analytical balance and transferred into the silica crucible. Placed the crucible in the muffle furnace and ignited the sample until the sample was completely burnt and only ash remains. Allowed the crucible to cool to room temperature. Weighed the crucible and ash using an analytical balance.Repeated until a constant weight is obtained [7].

Total Ash Value (%) = (Weight of Ash / Weight of Sample) x 100

Foam test: 5ml of reetha extract was taken in a test tube. Few drops of sodium bicarbonate was added to it, shaken vigorously and kept for three minutes and observed how long the form persists[8].

Castor Oil

Determination of Acid Value: A clean conical flask of 250 ml capacity was taken. 12.5ml each of ether and alcohol was added to it. Added one drop of phenolphthalein and neutralized using 0.1N KOH. Then 5g of accurately weighed castor oil was added to it. Added one drop of phenolphthalein and titrated using 0.1N KOH until solution remains faintly pink for 30 seconds after shaking[8].

Acid value =5.61n/w

Where, n = number of ml of 0.1 M potassium hydroxide required

w = weight in g of castor oil

Determination of density: Take a 25 ml specific gravity bottle which was cleaned and dried properly. Then weighed the empty, dry specific gravity bottle with its stopper (W1). After that filled the specific gravity bottle with distilled water of the extract filtrate on addition of Mayer's reagent was taken as evidence of the presence of alkaloids in the extract.[37]

Hager's test: 2ml of Hager's reagent was added to a little of the aloe vera extract. Yellow precipitate confirmed the presence of alkaloids

at 25°C. Then, wiped off any excess water and weighed the specific gravity bottle with water (W2). Again, made the specific gravity bottle empty and clean, then filled it with castor oil at 25°C. After that weighed the specific gravity bottle with castor oil (W3)[9].

Flax seed

Confirmatory Test for Fatty Acids

Bromine water test: 1ml of the flaxseed extract was taken in a test tube. 1-2ml of bromine water was added to the sample. Shaken gently. The discoloration of the solution confirmed the presence of fatty acids [35].

Copper soap test: 1-2ml of flaxseed extract was taken in a test tube. A few drops of 10% copper sulfate solution was added to the sample. 1-2ml of NaOH solution was then added and shaken well. The formation of blue precipitate confirmed the presence of fatty acids.

Emulsion test: The flaxseed extract was added to water taken in a test tube and shaken vigorouslyA drop of soap solution was added. Foam was produced and it was stable for 3 minutes which confirmed the presence of fatty acids [11].

Saponification test: 2ml of extract was mixed with NaOH solution in a beaker and heated. Then it was allowed to cool. After that dilute HCl was added drop-wise to the mixture. An oily layer was formed which confirmed the presence of fatty acids [11].

Aloe vera

Confirmatory Test for Alkaloids

Dragendorff 's test: To 1 ml of the aloe vera extract taken in a test tube, few drops of Dragendorff's reagent was added. A prominent yellow precipitate confirmed the test as positive.

Wagner's test: Few drops of Wagner's reagent was added along the side of the test tube to 1 ml of extract. A reddish-brown precipitate confirmed the test as positive[11].

Mayer's test: 1 ml of the aloe vera extract was stirred with 5 ml of 1% HCL on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with a few drops of Mayer's reagent. The turbid

Confirmatory Test for Flavonoids

Ammonia test: A few drops of 1% NH3 solution was added to 1 ml of the aloe vera extract in a test tube. A yellow coloration was observed which confirmed the presence of flavonoids[11].

Sodium hydroxide test: Few drops of 20% NaOH solution was added to 1 ml of the aloe vera extract

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taken in a test tube. On addition of HCl, the changed yellow colour of the extract turned to a colourless solution that depicted the presence of flavonoids[11].

Preparation of herbal hair serum

Preparation of oil phase: Mixed castor oil, 1 ml vitamin E and 1 ml tween 20 in a mortar. Stirred for 15 minutes.

Preparation of aqueous phase: In a china dish, added aloe vera extract, fenugreek extract, 1 ml orange-peel extract and 2 ml reetha extract. Mixed well and added flaxseed extract. Stirred for 15 minutes. Added the aqueous phase into the oil phase drop wise and mixed until a homogenous system was obtained. 0.4ml methyl paraben was added as preservative and 1ml orange oil was added for imparting fragrance. Made up the volume up to 15ml using distilled water and mixed well.[12]

Table1. Formulaforherbal hairserum

INGREDIENTS	FORMULATION F1 (15ml)	FORMULATION F2 (15ml)	FORMULATION F3 (15ml)	FORMULATION F4 (15ml)
Flaxseed extract	1ml	2ml	3ml	4ml
Aloevera extract	1ml	2ml	3ml	4ml
Reetha extract	2ml	2ml	2ml	2ml
Fenugreek extract	0.5 ml	1ml	2ml	3ml
Orangepeel extract	1ml	1ml	1ml	1ml
Castoroil	1ml	2ml	3ml	4ml
Vitamin E	1ml	1ml	1ml	1ml
Tween20	1ml	1ml	1ml	1ml
Methylparaben	0.4ml	0.4ml	0.4ml	0.4ml
Orangeoil	0.6ml	0.6ml	0.6 ml	0.6ml
Distilled water	q.s	q.s	q.s	q.s

Evaluation studies

Physical appearance: Physical appearance was evaluated by observing the texture, colour and smell of the formulated serum [13].

pH: The pH of the formulation was determined by using pH meter and the results are shown in table IV.

Homogeneity test:

A glass slide was washed, cleaned and dried. Then the glass slide was smeared with the hair serum and sealed with acover glass. After that the slide was placed under a light source and presence of coarse particles/homogeneity was investigated. Herbal hair serum was tested by usual examination for homogeneity and tested for lumps, flocculates or aggregates [13].

Sterility test: Agarmedium was prepared and sterilized.Transferred the medium into petridishes which were previously sterilized at 150^0C in a hot air oven and left the agar to solidify.Each of the formulations were added in the holes drilled in the agar medium. Simultaneously, the same procedure was carried out with a standard which containsEscherichia coli. The petridishes were incubated for 48 hours at 37^0C and after incubation, checked whether the bacterial colony is present or not [14].

Sensitivity test: conducted patch test for checking sensitivity. Selected a small, discrete area of skin, i.e, inner elbow. Washed the selected area with mild soap and water and then patted dry with a clean towel. Taken a small amount of the serum and applied to the test area. The test area should not be rubbed. It was kept undisturbed for 15 minutes. [15]

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Spreadability study:

Four filter papers were taken which was made in the shape of a square for the convenience of calculation. 3ml of hair serum was added dropwise at the centre to each of the filter paper and waited for 10 minutes to spread. The total area (A1) of the filter paper and the area of the hair serum that was spread (A2) were measured [16].

% Spread by area = (A2/A1)x100

Stability study: The herbal hair serum formulations were kept in a closed container for one month at room temperature for testing the stability of the prepared formulation. The parameters such as colour, pH, homogeneity, spreadability, appearance and skin irritation were evaluated.

III. RESULT AND DISCUSSION

Preformulation studies

TableII. Results of preformulation study

INGREDIENT	TEST	RESULT
Reetha	Losson drying	Moisturecontentisfoundto be 0.234 % whichcomes under the limit ≤ 12%
	pH	pHwasfoundto be6 which comesundertheidealrange of 4.5-7.5
	Saponintest	Foampersistfor3minutes confirms the presence of saponin.
	Totalashvalue	Ashvaluewasfoundto be 0.0505%whichcomesunder the limit ≤5%.
flaxseed	Brominewatertest	Discolorationoccursconfirm the presence of fatty acid
	Coppersoaptest	Formationofblueprecipitate confirm the presence of fatty acid
	Emulsiontest	Theproducedfoamstablefor 3 minutes confirm the presence of fatty acid
	Saponificationtest	Anoilylayerisformed confirms the presenceoffattyacid
Aloevera	Testforaloin	No orange colour is produce indicates the absence of aloine
	Dragendroff'stest	No orange or reddish brown precipitate is formed confirms the absence of alkaloid
	Wagner'stest	No reddish precipitate is formed confirms the absence of alkaloid
	Hager'stest	No yellow precipitate is formed confirms the absence of alkaloid
	Mayer'stest	Nocreamy/ yellow precipitate formed which confirms the absence of alkaloid.
Castoroil	Acidvalue	Acid value is found to be 0.4488 which comes under the range of 0.14-4.7
	Density	Densityisfoundtobe 0.952g/cm^3

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The preformulation studies were conductedto confirm the presence of various chemical constituentsin the major ingredients used for the formulation. The results of the study proved that all these ingredients contains constituents which give desired properties for the hair serum. The presence of aloin in aloe vera causes skin irritation so it is made sure that no aloin content was present in the hair serum, as confirmed by tests conducted.

Organoleptic properties evaluation test: The organoleptic properties of formulated hair serum were judged by colour, texture and odour.

Table III. Evaluation of Formulation for Organoleptic Properties

Evaluation parameters	Formulation F1	Formulation F2	Formulation F3	Formulation F4
Colour	Milkywhite	Milkywhite	Milkywhite	Yellowish white
Texture	Smooth	Verysmooth	Verysmooth	Smooth
Odour	Fruity	Fruity	Fruity	Fruity

Formulation F1 and F4 were less smooth compared to formulation F2 and F3. Comparing the organoleptic properties formulation F2 and F3was found to be best because it has very smooth texture and milky white colour.

Measurement of pH: The pH of formulated hair serum was recorded in the table.

Table IV. pH of the formulation

Formulation	pH
FormulationF1	6
FormulationF2	5
FormulationF3	5
FormulationF4	5

pH of formulated hair serum was slightly acidic, promoting healthy hair by keeping the cuticle closed and minimizing damage. Comparing the pH of the formulated hair serum formulation F2, formulation F3and formulation F4 were found to be in the ideal range of 4.5-5.5. Formulation F1 does not come under this range

Test of Spreadability: Prepared hair serum was tested for ability of spreading. The result of spreadability tests were listed below.

Table V. Results of spreadability study

Formulation	Spreadability
FormulationF1	13.502%
FormulationF2	9.495%
FormulationF3	18.349%
FormulationF4	14.197%

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Among all the formulations, formulation F3 have greater spreadability compared to others. The order of spreadability was found to be formulation F3 > formulation F4 > formulation F1 > formulation F2.

Skin Irritation test:Amongall theformulatedhairserums,noneshowed anytypeofskin irritation.

Test for homogeneity: The formulated hair serums F1, F2, F3 and F4 are tested for homogeneity and all the formulations were found to be homogeneous.

Test for sterility: Microbial growth was present in the test standard containing Escherichia coli. All the formulations were tested no microbial growth was found, it indicates that the amount of preservative added was accurate for the preparation and the formulations are sterile.

Stability study of formulations:

All the formulations were evaluated for their stability. Stability studies of a formulation can be defined as time from date of manufacture and the packaging of the formulation, until its chemical and biological activities not less than predetermined level of labelled potency and physical characteristics have not changed appreciably. Stability studies were conducted for 30 days under room temperature to understand changes occurring to the formulation during that period.

Table VI. Evaluation of Stability of Formulated Hair Serum

Parameter	FormulationF1	FormulationF2	FormulationF3	FormulationF4
Colour	Paleyellow	Slightlyyellow	White	Yellow
pH	Nochange	Nochange	Nochange	Nochange
Spreadability	+++	+++	++++	+++
Fragrance	Fruitysmell ++	Fruitysmell +++	Fruitysmell +++	Fruitysmell ++++

Room temperature: 25-30^{0}C, N- No change, +Good, ++Very good, +++ Excellent

From the study it was concluded that, after conducting the stability studies of all the 4 formulations, there was no change in colour, fragrance, pH and spreadability over a period of 30 days. From all the formulated hair serums, formulation F3 gives an appealing colour, odour and very smooth texture even after keeping it for several weeks while other formulations show change in theircolour and odour. Formulation F3has the greater spreadability compared to other formulations. So, it is easy to apply and is more effective. So formulation F3 was selected as the best among the four formulations and hence it was used for conducting further studies.

Evaluation and Comparison of final formulation with marketed formulation:

Table VII. Comparison of final formulation (F3) with marketed formulation

Evaluation parameter	**FormulationF3**	**Marketed hair serum 1(Moha hair serum)**	**Marketedhairserum 2 (Elois hair serum)**
Colour	Milkywhite	Transparent yellow	Transparent
pH	5	5	6
Skinirritationtest	Noirritation	Noirritation	Noirritation
Fragrance	fruitysmell	Fruitysmell	Soapysmell

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Appearance	Verysmooth	Verysmooth, viscous	Verysmooth
Spreadability	++++	+	+++
Homogeneity	No lumpsor aggregation	No lumps oraggregation	No lumpsor aggregation

It was concluded that the formulation F3 and the marketed herbal serums were having almost similar properties. Formulation F3 stands out when compared to marketed hair serum 1 (Moha hair serum containing flax seed oil, hibiscus oil, water lily oil) and marketed hair serum 2 (Eloishair serum containing aloe vera oil, argan oil, vitamin E) considering overall properties. The formulation F3 was found to be the best among the two marketed hair serums because of its greater spreadability.

IV. SUMMARY AND CONCLUSION

The herbal hair serum is successfully formulated using various natural ingredients such as flaxseed (Linumusitatissimum), fenugreek (Trigonellafoenum-graceum), Aloe Vera (Aloe barbadensis Mill), orange peel (Citrus sinensis), reetha (Sapindusmukorossi) and castor oil (Ricinuscommunis). The pre-formulation study of ingredients such as flaxseed extract, aloe vera extract, reetha extract and castor oil were tested and then confirmed for the presence of various chemical constituents. This study proved that all these ingredients contains constituents which give desired properties for the hair serum. The presence of fatty acids in flax seeds are responsible for imparting antioxidant properties. The surfactants present in reetha offers cleansing action. Also the presence of aloin in aloe vera causes skin irritation so it is made sure that no aloin content was present in our hair serum.From the studies it was concluded that this formulated herbal hair serum F3 provides a better option as it contains least side effect and also have antioxidant property. The formulated herbal hair serum was evaluated for various parameters such as physical appearance, pH, skin irritation test, fragrance, stability and spreadability.The prepared herbal hair serum is claimed for various purposes such as hair growth, conditioning, cleansing and moisturization. The ingredients such as flaxseed, fenugreek and castor oil have the ability to enhance hair growth as these ingredients contains alpha-linoleic acid, protein and ricinoleic acid respectively. Some ingredients were found to have common properties which in turn adds to the efficiency of the hair serum, as in the antioxidant properties of flax seeds and aloe vera as well as the hair growth stimulating properties of fenugreek and castor oil. Among the four formulations, formulation F3 was found to be the best and more stable as per the results of evaluation test, the final selected formulation (formulation F3) was compared with two marketed herbal hair serums (having similar ingredients as that of formulation F3) for their various properties.From this study it was concluded that all the ingredients used to formulate the herbal hair serum were safe and effective. Also formulation F3 was found to be advantageous over the marketed formulations because of its greater spreadability. Future studies using in-vivo models will be crucial in translating this finding to human beings.

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