ORIGINAL ARTICLE

Formulation and Evaluation of Polyherbal Hair Serum

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Abstract

Aim: This project was established to develop and test a polyherbal hair serum based on the use of plant extracts, which are safer and more effective to address hair loss as an alternative. **Materials and Methods:** *Nardostachys jatamansi*, *Wrightia tinctoria*, *Eclipta prostrata*, *Cuscuta reflexa*, *Rubia cordifolia*, *Phyllanthus emblica*, *Plectranthus amboinicus,* and *Pterocarpus santalinus* were extracted by the hot method and cold method to obtain the plant materials. Thereafter, we prepared four various formulations (F1-F4) using ingredients, such as Tween 80, Propylene Glycol, Essential oils, Almond oil, and Sodium Benzoate. We examined physical characteristics, such as color, scent, Type of emulsion, PH, viscosity, spreadability of the substance, and the skin irritation properties. A rapid phytochemical analysis was also done to determine the major secondary metabolites present. **Results:** Among all the formulations, F4 was the best one. It appears to be shiny gold-yellow, it smells pleasant, is stable as an oil-in-water emulsion, the pH is 4.89, the viscosity is approximately 1284.6cP, it spreads easily, and it does not irritate the skin. The phytochemical test revealed it contains sugars, alkaloids, glycosides, tannins, saponins, proteins, and fixed oils, indicating that it is loaded with phyto-nutrients with anti-oxidant and hair growth promotion properties. **Discussion:** The optimized formulation (F4) showed stable physicochemical characteristics and good cosmetic acceptability, indicating successful emulsification and formulation design. The presence of bioactive phytoconstituents suggests potential synergistic effects in promoting scalp health and supporting hair growth. **Conclusion:** The formulated polyherbal hair serum had good physical properties and possible health benefits. It is a safe, secure, and esthetically pleasing choice to people who are in the struggle with hair loss. However, more *in vivo* and clinical studies are required to establish its safety and efficacy in the long run.

Key words: Alopecia, herbal formulation, natural therapeutics, oil-in-water emulsion, physicochemical evaluation, phytochemical screening, polyherbal hair serum

INTRODUCTION

Hair is one of the most important characteristics of mammals, since it is involved in a wide range of functions, such as thermoregulation, physical protection, dispersion of sweat and sebum, sensory and tactile functions, and social interactions, protecting the body against heat, cold, sunlight, pollution, injuries, and impacts.[1,2] Human hair is a filamentous biomaterial mainly composed of α-keratin (~80%), a Sulfur-rich protein that provides strength and stability through disulfide bonds. Structurally, hair has three parts: Cuticle, cortex, and medulla. The cuticle is the outer protective layer responsible for smoothness and shine, while the cortex forms the bulk of the hair and determines its color and mechanical properties. The medulla, present mainly in

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coarse hair, may contribute to insulation and hair volume.[3,4] Hair fibers are mainly composed of 65–95% of protein and may also contain 30–35% of water by weight. The remaining components include lipids, pigments, and other minor substances. The major amino acids present in hair are glycine, threonine, aspartic acid, glutamic acid, lysine, cysteine, and tyrosine. As a result, the chemical properties of human hair are largely determined by α-keratin.[5,6] Hair follicles form during embryonic development through interactions between epidermal stem cells and dermal mesenchyme, and then undergo a repeating cycle of growth (anagen), regression (catagen), and rest (telogen). Anagen is the active growth phase, catagen is a short transitional phase with follicle shrinkage, and telogen is the resting phase, followed by hair shedding.[7-9]

Alopecia is a condition characterized by thinning and loss of hair from the scalp or other body parts due to factors, such as genetics, stress, hormonal imbalance, or medications. Normally, 50–100 hairs are shed daily and replaced, but failure of regrowth leads to temporary or permanent hair thinning and baldness.

A hair serum is a liquefied or silicone-based hair care and styling product that is lightweight in nature and coats the surface of hair strands to create a protective layer. It relaxes frizz, increases luster, makes hair easy to handle, and shields hair against environmental aggressors, such as heat, humidity, and pollution. An herbal hair serum is a liquid, leave-in hair care product of typically plant-based ingredients, such as herbal extracts, essential oils, and botanical nutrients. It is created to be nourishing and strengthening to hair, frizz-mitigating, adding shine, damage protective, and having an overall good texture to hair, but without harsh chemicals used. Herbal hair serums are generally lightweight, non-greasy, and are to be applied on the damp or dry hair to help control hair and its health.[10]

The selection of herbal ingredients, such as Jatamansi, Bhringraj, Amla, and Wrightia was based on their well-documented hair growth–promoting, antioxidant, and scalp-nourishing properties. Different extraction methods, including hot and cold extraction, were employed depending on the nature of phytoconstituents to ensure maximum yield and stability. Ethanol–water ratios of 40:60 and 60:40 were chosen to efficiently extract both polar and non-polar compounds. The extracts were standardized based on consistency, appearance, and concentration before formulation. Excipients, such as Tween 80 and propylene glycol, were selected for their solubilizing and moisturizing properties. Multiple formulations (F1-F4) were prepared to optimize parameters, such as pH, viscosity, and spreadability. The optimized formulation (F4) showed a pH close to that of the scalp, ensuring compatibility and reduced irritation. Preliminary stability observations indicated acceptable physical stability of the serum. The developed formulation showed comparable physicochemical properties with marketed herbal serums. Further *in vivo* studies and clinical trials are planned to validate its efficacy in promoting hair growth.

Causes of hair loss

Androgenetic alopecia, Anagen effluvium, Alopecia areata, Trichotillomania, Loose anagen hair syndrome, hereditary hair loss, age, cancer treatment, hormonal imbalance, hair care, scalp psoriasis, sexually transmitted diseases/infections, thyroid disease, malnutrition.[11] The synthetic drugs and their action were mentioned in Table 1.

Natural ingredients offer a safer and more holistic alternative to synthetic drugs used in hair loss management, which are often associated with hormonal, cardiovascular, and scalp-related side effects. The present formulation contains botanicals, such as Jatamansi, Bhringraj, Amla, *Cuscuta reflexa*, *Wrightia tinctoria*, Manjistha, Fenugreek, Karpooravalli, and Red sandalwood, which are rich in antioxidant, anti-inflammatory, and follicle-stimulating phytoconstituents. These herbs act through multi-target, gentle mechanisms that protect hair follicles from oxidative stress and inflammation, improve microcirculation, and support scalp health without significantly affecting systemic hormones or blood pressure.

In addition to promoting hair growth, these natural ingredients enhance scalp barrier compatibility and improve hair fiber quality by providing essential nutrients, vitamins, and minerals that strengthen the shaft and reduce breakage. Their predominantly local action minimizes endocrine disturbances and systemic toxicity, making them suitable for long-term use, especially in younger individuals. Overall, the mild side-effect profile and better cosmetic acceptability of herbal formulations improve patient safety, confidence, and treatment compliance compared to conventional synthetic therapies.

MATERIALS AND METHODS

The materials that are used for this research were procured from certified agencies and taxonomically identified for authenticity. The scientific description of each herb was mentioned in Table 2.

Methods

Selection of materials

To the recent diseases and disorders, loss of hair (Alopecia areata) is a great and major issue. The synthetic chemicals used in health are numerous and have major side effects. To minimize this effect on human health, different natural herbals are consulted under several monographs and a handful of herbs.

Table 1: Synthetic treatment to hair loss

Drug	Mechanism of action	Disadvantages	Side effects
Minoxidil	Opens potassium channels, which increases scalp blood flow and growth factors (VEGF, PGE_2)	Continuous use needed, Scalp and cardiac issues	Dizziness, edema, fast heartbeat
Finasteride	Selectively inhibits type II 5-α reductase. Reduces conversion of testosterone to DHT, preventing follicle miniaturization.	Hormonal effects, unsafe in pregnancy	Low libido, gynecomastia, depression
Dutasteride	Inhibits type I and type II 5-α reductase. Produces greater suppression of DHT than finasteride	Stronger hormonal suppression	Sexual dysfunction, gynecomastia, depression
Deuruxolitinib	JAK-1 and JAK-2 inhibitor, Suppresses JAK-STAT pathway, reducing autoimmune inflammation in alopecia areata	Immunosuppression, monitoring required	Infections, headache, acne, and blood clots

VEGF: Vascular endothelial growth factor, DHT: Dihydrotestosterone, PGE_2: Prostaglandin E_2

Table 2: List of herbals used for the formulation of herbal hair serum

S. No.	Name	Biological source	Family	Chemical constituents
1.	Jatamansi	Dried roots and rhizome of *Nardostachys jatamansi*	Valerianaceae	Jatamansone, Jatamansic acid, β- sitosterol
2.	Wrightia	Stem, bark, and leaves of the plant *Wrightia tinctoria*	Apocynaceae	Indigotin, Indirubin, β-Amyrin, α-Amyrin, β-Sitosterol, Rutin, Quercetin, Polyphenols, Glycosides.
3.	Karpooravalli	Dried whole plant of *Plectranthus amboinicus*	Lamiaceae	Carvacrol, Thymol, Rosmarinic acid, Quercetin, Luteolin, Rutin.
4.	Bhringraj	Fresh or dried whole plant or leaves of *Eclipta prostrata* L.	Asteraceae	Wedelolactone, Ecliptine, Demethylwedelolactone.
5.	Arambel	Dried whole plant (stems) of *Cuscuta reflexa* Roxb	Convolvulaceae	Kaempferol, Myricetin, β-Sitosterol, Quercetin, Lupeol.
6.	Manjistha	Dried roots and rhizomes of *Rubia cordifolia* L. (syn. Rubia manjitha roxb.	Rubiaceae	Purpurin, Alizarin, Munjistin, Rubiadin.
7.	Amla	Fresh or dried pericarp (fruit) of *Phyllanthus emblica* L.	Phyllanthaceae	Gallicacid, Emblicanin A, Emblicanin B, Ellagic acid, Vitamin C, quercetin, Phytosterols.
8.	Red sandalwood	Heartwood of *Pterocarpus santalinus* Linn	Fabacea	Dibenzylbutane/ furofuran lignans, Calocedrin, eudesmin, marsupsin, pterosupin, neoflavones I & II and other isoflavonoids. The list of herbals used for the formulation of herbal hair serum were showed in figure 1- 18

- *Nardostachys jatamansi* (Jatamansi)
- *W. tinctoria* (*W. tinctoria* or Sweet Indrajao)
- *Coleus aromaticus* (Karpooravalli or Indian Oregano)
- *Eclipta prostrata* (Bhringraj or False Daisy)
- *C. reflexa* (*C. reflexa* or Dodder)
- *Rubia cordifolia* (Manjistha or Indian Madder)
- *Phyllanthus emblica* (Amla or Indian Gooseberry)
- *Pterocarpus santalinus* (Red sandalwood or Rakta Chandana).

To prepare a serum, the excipients are:

- Lavender oil
- Rosemary oil
- Tea tree oil
- Almond oil
- Tween 80
- Propylene Glycol
- Sodium benzoate.

Collection of materials

The materials that are used for this research were purchased from a certified local market and taxonomically identified for authenticity. Assessment examinations are conducted for the herbal extract, as they say in their description. The above table indicates the source of purchase.

Solubility studies

The solubility studies were performed as per IP guidelines, 1 g of each powder was weighed and taken in a round-bottom flask and the required quantity of the solvent was taken

into the flask and stored in a room having a temperature of 25°C. An additional amount of solvent was added gradually until each powder dissolved completely. The volume of the solvent was noted. This method was carried out with various solvents, such as water and organic solvents, such as methanol, ethanol, and hydro-alcoholic solutions, such as ethanol: water in different ratios.

The above-listed powders, except amla, show free solubility in hydro-alcoholic solution, such as ethanol:water (40:60). The amla powder shows free solubility in hydro-alcoholic solution, such as ethanol:water (60:40).

Extraction of active components

The chemical constituent's extraction was made in two major processes.

Hot extraction process

In this process, water-soluble substance, 1 g of Alma is weighed, and approximately 25–30 mL of ethanol: water (60:40) is added to the powder. Boil the solution for at least 30–40 min in a condenser. Then allow the solution to cool down after the required time of condensation and filter it using a muslin cloth or triple-layered filter paper. The filtrate was concentrated Amla solution.[12]

Cold extraction process

In this process, the hydro-alcoholic soluble components, such as Jatamansi, *W. tinctoria*, Karpooravalli, Bhringraj, *C. reflexa*, Manjistha, and red sandal wood, were weighed 1 g each, and 200 mL of Ethanol: water (40:60) was added, that is, (120 ml water and 80 mL Ethanol) to the powders in a round-bottom flask. Macerate the solution between 5 and 6 days, and the extract should be stirred twice/thrice a day. Filter the extract twice using a muslin cloth or triple-layered filter paper after maceration. The filtrate was kept for evaporation in the water bath until the herbal extract solution was concentrated.[12] All the above solutions were combined to obtain a uniform total herbal extract.

Phytochemical tests for herbal extract

Test for carbohydrates

Molish test

Take 2 mL of the test solution in a test tube and add 2–3 drops of Molish reagent. Carefully add concentrated sulfuric acid along the side of the test tube without shaking. Formation of a violet or purple ring at the junction indicates the presence of carbohydrates.[13]

Iodine test

Take 2 mL of the test solution and add 2–3 drops of iodine solution. The appearance of a blue-black color confirms the presence of starch.[13]

Barfoed's test

Take 2 mL of the test solution and add 2 mL of Barfoed's reagent. Heat the mixture in a boiling water bath for 1–2 min. Formation of a red precipitate indicates the presence of monosaccharides.[13]

Test for alkaloids

Dragendorff's test

Take 2 mL of the acidic test solution and add a few drops of Dragendorff's reagent. Formation of an orange or reddish-brown precipitate shows the presence of alkaloids.[14]

Mayer's test

Take 2 mL of the acidic test solution and add a few drops of Mayer's reagent. Appearance of a cream or white precipitate indicates the presence of alkaloids.[14]

Test for glycosides

Borntrager's test

Boil the test solution with dilute sulfuric acid, cool, and extract with chloroform. Separate the chloroform layer and add ammonia solution. Formation of a pink or red color indicates the presence of anthraquinone glycosides.[15]

Keller–Killiani test

Take 2 mL of the test solution and add glacial acetic acid containing ferric chloride. Carefully add concentrated sulfuric acid along the side of the test tube. Formation of a brown ring at the junction indicates the presence of cardiac glycosides.[15]

Test for tannins

Ferric chloride ($FeCl_3$) test

Take 2 mL of the test solution and add a few drops of 5% $FeCl_3$ solution. Development of a blue, green, or black color confirms the presence of phenols or tannins.[16]

Lead acetate test

Take 2 mL of the test solution and add a few drops of lead acetate solution. Formation of a white or yellow precipitate indicates the presence of tannins.[16]

Test for saponins

Foam test

Take 2 mL of the test solution in a test tube and shake it vigorously for about 2 min. Formation of stable, persistent foam indicates the presence of saponins.[17]

Emulsion test

Take 2 mL of the test solution and add a small quantity of ethanol, and shake well. Add water to the mixture. Formation of a white milky emulsion indicates the presence of fixed oils or fats.[17]

Test for proteins

Biuret test

Take 2 mL of the test solution, add 1 mL of sodium hydroxide solution, and then add a few drops of copper sulfate solution.

Formation of a violet or purple color confirms the presence of proteins.[18]

Test for amino acids

Xanthoproteic test

Take 2 mL of the test solution and add concentrated nitric acid, and heat gently. After cooling, add sodium hydroxide solution. Development of a yellow to orange color indicates the presence of aromatic amino acids.[19]

Ninhydrin test

Take 2 mL of the test solution and add a few drops of ninhydrin reagent, and heat in a water bath. Formation of a blue or purple color indicates the presence of amino acids.[19] The results of phytochemical tests are mentioned in Table 3.

Formulation of hair serum

The hair serum formulation was made by weighing the ingredients accurately and separating them into two phases, that is, the aqueous phase and the oil phase, then take them in two separate beakers marked with clear labels. Both the beakers were placed in a water bath and heated around 70°C simultaneously, and the temperature of the components was constantly monitored using a thermometer to ensure uniformity. The beakers were taken out of the water bath and allowed to cool to 40°C under controlled conditions to avoid instability of the formulation.

The aqueous phase was then placed in a water bath to cool and kept under a mechanical stirrer,[12] which was first rotated at a slow speed. Add drop-wise solution of oil phase to the aqueous phase and stir continuously by gradually increasing the speed of the mechanical stirrer for about 30–40 min until a uniform stable emulsion is formed. Ten minutes before final completion of the mixing process, the necessary amount of preservative was added to the emulsion and left to mix well by continuous stirring to improve microbial stability and improve the shelf life.

After the preparation of the emulsion, the prepared serum was left without any disturbance at room temperature for about 30 min to further stabilize the emulsion. Sonication[11] of the formulation was then performed at 30–40 min with the occasional stirring by hand to maintain equal distribution of energies. Sonication aided in the decrease of the particle size, which increased the stability and effectiveness of penetration of the hair serum. At last, the completed product was moved into clean and dry containers, which are correctly labeled with the required information and preserved in a cool and dry place to avoid direct sunlight penetration for the prevention of product deterioration. Different formulations were prepared to identify the perfect hair serum, and they are mentioned in Table 4.

Evaluation

Physical evaluation

1. Color: The color of the formulation was observed through the naked eye. It was done at the bright white light source.[20]
2. Odor: The formulation was taken to the odorless area. Then it was taken into a glass slide, and the odor of the sample was identified by 2–3 people.[20]
3. Type of emulsion: The formulation was taken into a beaker, which was cleaned and dried. It was taken into a dark room having a bright white light source. The formulation was kept in a slant position in front of the light source. Then the formulation was observed for the light reflection. Based on the light reflections, the formulation can be categorized into two types of emulsions.

Table 3: Results of phytochemical tests for the extract

Type of test	Observation	Inference
Molish test	Formation of a violet ring at the junction	Presence of carbohydrates
Iodine test	Formation of a blue-black color	Presence of Polysaccharides
Barfoed's test	No formation of red precipitate occurred	Absence of monosaccharides
Dragendorff's test	Formation of an orange precipitate	Presence of alkaloids
Mayer's test	Formation of a cream precipitate	Presence of alkaloids
Borntrager's test	No red or pink color was formed	Absence of anthraquinone glycosides
Keller–Killiani test	Formation of a violet ring at the junction	Presence of cardiac glycosides
Ferric Chloride ($FeCl_3$) test	A greenish black color is appeared	Presence of condensed tannins
Lead acetate test	Formation of a white precipitate	Presence of tannins
Foam test	Formation of stable, persistent foam	Presence of saponins
Emulsion test	Formation of a white milky emulsion	Presence of fixed oils or fats
Biuret test	Formation of a violet color	Presence of proteins
Xanthoproteic test	Formation of a yellow to orange color	Presence of aromatic amino acids
Ninhydrin test	No purple or blue color formed	Absence of free amino acids

a. Oil in water type (O/W): The oil particles in the emulsion appear as spherical-shaped globules and reflect the light. The background was dull because most of the emulsion was occupied with water, and water does not reflect the light as much as oil.
b. Water-in-oil type (W/O): Most of the emulsion was occupied by oil, and a low amount of emulsion was occupied with water. Hence, the oil phase of the emulsion reflects the light in high amount. The water phase of the emulsion shows low light as spherical-shaped globules.

1. pH: pH is defined as the negative logarithm of the hydrogen ion activity in a solution. It represents the degree of acidity or alkalinity of a formulation and is determined potentiometrically using a calibrated pH meter equipped with a suitable electrode system under controlled temperature condition.[20]
2. Viscosity: Viscosity is defined as the measure of a fluid's internal resistance to flow when subjected to an applied force. It indicates the rheological behavior of a formulation and is determined using a Brookfield viscometer at specified temperature and shear conditions.[20]
3. Spreadability: Spreadability is defined as the ability of a semi-solid or liquid formulation to spread evenly over a surface when a certain force is applied to it. This feature shows how smoothly the topical preparations could be used and affects how patients accepted those.[20]
4. Skin irritation test: A skin irritation test refers to a type of evaluative test used to determine the possibility of a given formulation to cause reversible inflammatory changes, such as erythema or edema, following topical application of the formulation to the skin under identified test conditions.[21]

Table 4: Composition of various formulations regarding the herbal hair serum

Components	Quantity (mL)			
	F1 (Qty: 30 mL)	F2 (Qty: 30 mL)	F3 (Qty: 60 mL)	F4 (Qty: 60 mL)
Aqueous phase				
Herbal extract	10 mL	10 mL	20 mL	23 mL
Water	10 mL	7 mL	14 mL	--
Tween 80	3 mL	6 mL	12 mL	17 mL
Propylene Glycol	2 mL	2 mL	4 mL	9 mL
Sodium benzoate	--	--	--	0.1 mL
Oil phase				
Lavender oil	1.25 mL	1.25 mL	3.33 mL	3.33 mL
Rosemary oil	1.25 mL	1.25 mL	3.33 mL	3.33 mL
Almond oil	1.25 mL	1.25 mL	3.33 mL	3.33 mL
Tea tree oil	1.25 mL	1.25 mL	0.1 mL	0.1 mL

RESULTS

Physical evaluation results

The results of the physical evaluation are mentioned in Table 5 of four formulations of herbal hair serum.

DISCUSSION

Hair loss is a multifactorial and complicated disorder, which is determined by genetic, hormonal, nutritional, inflammatory, and environmental influences. There are three phases of the regular hair growth cycle: The growth phase (anagen), transitional phase (catagen), and the resting phase (telogen). Any disruption of this cycle, particularly in decreasing the

Table 5: Results for the physical evaluation of the formulations

Tests	F1	F2	F3	F4
Color	Light brown and cloudy	Cloudy and creamy yellowish brown	High creamy yellowish red	Clear golden yellow to amber, with a bright
Odor	Strong, pungent, irritant	Strong, pungent, irritant	Pleasant, mild	Pleasant and acceptable
Type of solution	Emulsion formed, but phase separation occurred	Emulsion of the O/W type was formed	O/W type of emulsion was formed	Emulsion of a perfect O/W type was formed
Spredability	Sticky and no spreadability	Good spreadability but watery in texture	Spreadability was low and scaly	Good spreadability, washable, and cooling effect
PH	3.5 (Weak acid)	8.3 (Weak basic)	7.5 (Neutral)	4.89 (Weak acid)
Viscosity	520 cP/5.20P	1920.1cP/19.201P	10275cP/10.275P	1284.6cP/12.846P
Irritation test	No irritation was observed	No irritation was observed	No irritation was observed	No irritation was observed

O/W: Oil in water type

Figure 1: *Nardostachys jatamansi*

Figure 2: *Wrightia tinctoria*

Figure 3: *Plectranthus amboinicus*

Figure 4: *Eclipta prostrata* L.

Figure 5: *Cuscuta reflexa* Roxb

Figure 6: *Rubia cordifolia* L

anagen period or increasing the telogen period, may cause thinning of the hair and excessive shedding. Oxidative stress, follicular inflammation, impaired scalp circulation, and hormonal imbalance in many individuals are major causes of follicular miniaturization and decreased hair density. Even though there are a number of synthetic pharmacological agents they can be employed in the management of alopecia, the long-term use of these drugs is often limited by adverse side effects and non-compliance of patients.

Most frequently prescribed drugs, such as minoxidil and finasteride, have their mechanisms of action either by increasing blood flow to the scalp or by altering hormonal pathways of follicular regulation. Although these agents might prove to be effective clinically, they are linked with the occurrence of side effects, such as scalp irritation, headache, hormonal imbalance, sexual dysfunction, and, in certain instances, cardiovascular complications. Since hair loss is usually a chronic disease that requires long-term medication, the safety profile of the therapy becomes the most important factor. This has led to mounting pressure to use safer and plant-based alternatives with therapeutic properties but with minimum systemic side effects.

Figure 7: *Phyllanthus emblica* L

Figure 10: *Rosmarinus officinalis*

Figure 8: *Pterocarpus santalinus* Linn

Figure 11: *Prunus dulcis*

Figure 9: *Lavandula angustifolia*

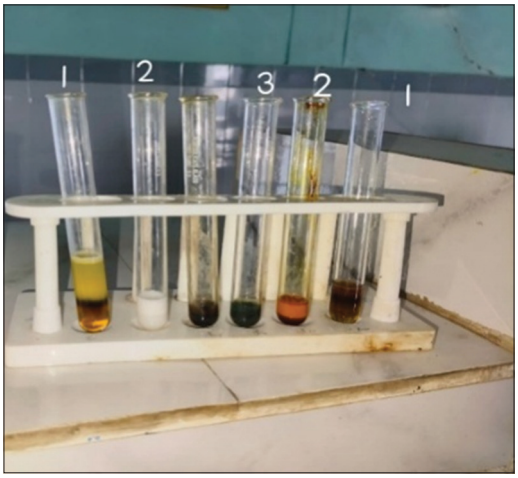

Figure 12: (From right) (1) Molish test, (2) Iodine test, (3) Barfoed's test, (From left) (1) Keller kiliani test, (2) Borntrager's test

A polyherbal hair serum was developed in this study, has the incorporation of some medicinal plants that are selected on the basis of antioxidant, anti-inflammatory, anti-microbial, and hair-growth-promoting capabilities. The optimized formulation (F4) had better physicochemical properties than the rest of the trial formulations (F1-F3). It had a clear golden-yellow look, pleasant smell, stable type of oil-in-water (O/W) emulsion, smooth spreadability, and did not indicate any phase separation. The pH of 4.89 was acceptable to apply on the scalp and thus compatible with the natural pH of the skin and low chances of irritation. The consistency of 1284.6 cP

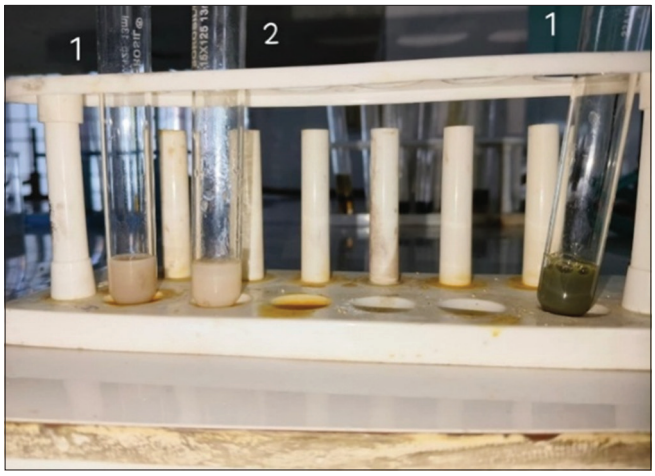

Figure 13: (Right) 1. $FeCl_3$ test. (Left), (1) Foam test, (2) Emulsion test

Figure 16: Represents formulation 4 which is a perfect herbal hair serum

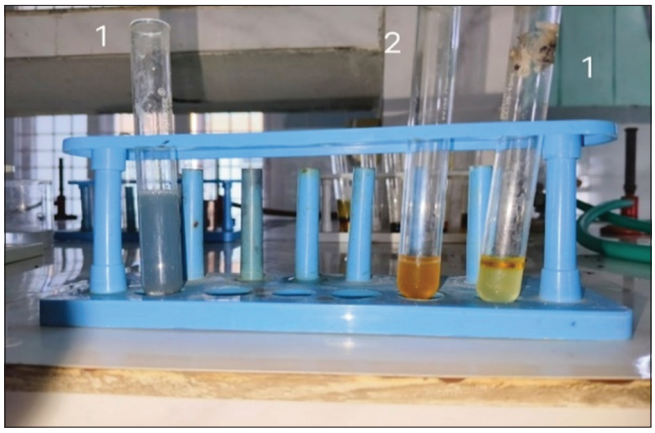

Figure 14: 1. Biuret's test (left). (Right) (1) Xanthoproteic test, (2) Ninhydrin test

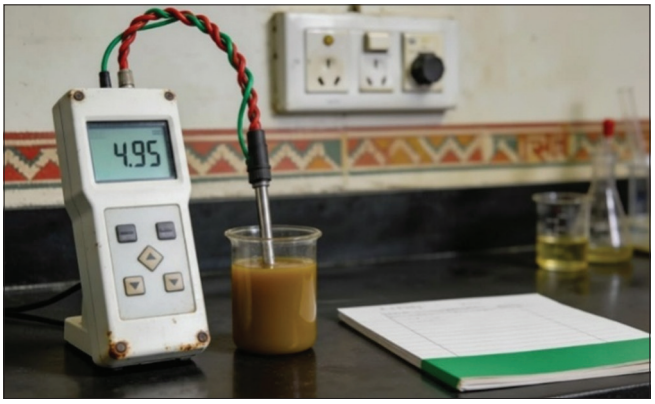

Figure 17: pH Test of the herbal hair serum

Figure 15: Represents formulation 1-3 indicates herbal hair serum combinations

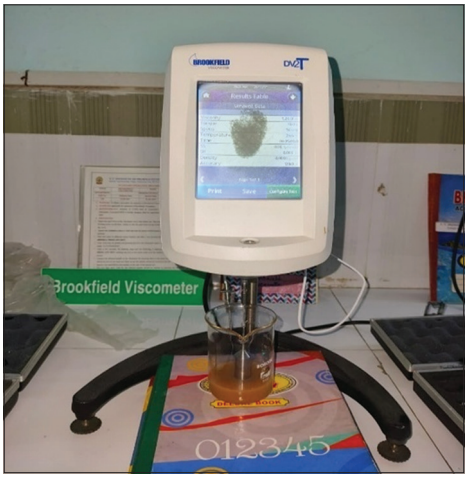

Figure 18: Viscosity test of the herbal hair serum

was also an indication of the appropriate consistency to be applied easily and to coat the hair strands effectively without being so sticky or watery.

The phytochemical analysis of the combined herbal extract contains carbohydrates, alkaloids, glycosides, tannins, saponins, proteins, and fixed oils. These biochemical compounds are very crucial toward scalp health and hair growth. Antioxidants are used to counter the free radicals that destroy the hair follicles, and the anti-inflammatory

agents suppress the irritation of the scalp and follicular stress. Tannins and saponins could help in the cleansing of the scalp and microcirculation, and the fixed oils could help in nourishing and strengthening the hair shaft. The combination of these phytoconstituents is known to yield a multi-transportation effect of these constituents in hair care, compared to synthetic drugs that can have only one effect.

In addition, the absence of skin irritation in all tested formulations is another suggestion of favorable dermatological safety. The use of natural ingredients increases patient acceptability because of its soft action, nice smell, and beauty. The herbal formulations are typically local actions on the scalp, and as such, there is minimal risk of systemic toxicity and hormonal interference. This property makes them suitable to use in the long run, especially in younger people and those who want to use them in preventing hair-care procedures.

All in all, the results of this research show that the formulated polyherbal hair serum, especially formulation F4, is stable, cosmetically acceptable, and could be useful in the treatment of hair loss. The formulation provides an alternative to synthetic treatments due to its ability to provide integral therapy and greater safety because it couples several plant extracts with complementary pharmacological actions. Nevertheless, despite the positive results of the preliminary physicochemical and phytochemical analysis, additional *in vivo* research and controlled clinical trials should be conducted to scientifically prove its prolonged safety and therapeutic effectiveness in humans.

CONCLUSION

The present research on the development and testing of a polyherbal hair serum shows a scientifically formulated, plant-based option to handle the problem of hair loss. Hair loss is a complex disease that is caused by hormonal, genetic, inflammatory, and environmental factors. Synthetic medications, such as minoxidil and finasteride, they are effective, and they often cause systemic side effects and restrictions on long-term use. The present study concentrated on a combination of medicinal herbs, where *N. jatamansi*, *E. prostrata*, *P. Emblica*, *W. tinctoria*, *C. reflexa*, *R. cordifolia*, *Plectranthus amboinicus,* and *P. santalinus* were identified as having antioxidant, anti-inflammatory, and follicle-stimulating properties.

Among the four formulations (F1-F4), formulation F4 exhibited good physicochemical properties, such as a stable O/W emulsion, an acceptable pH of 4.89, optimum viscosity of 1284.6 cP, good spreadability, and no skin irritation was observed. The screening of the phytochemicals showed that the composition contained carbohydrates, alkaloids, glycosides, tannins, saponins, proteins, and fixed oils, indicating high levels of bioactive components that could have a combination effect of improving the health of the scalp, strengthening hair fibers, and follicular activity.

In general, the optimized polyherbal hair serum is a cosmetically elegant, stable, and possible safe natural therapeutic agent in the treatment of alopecia. Its multi-targeted action and its positive safety profile justify its ability to be used in the long term. However, additional *in vivo* research and controlled clinical trials are needed to confirm its effectiveness in human subjects in the long run, safety, and therapeutic potential.

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