Development And Evaluation Of Polyherbal Formulation For Alopecia

[1]Chaitanya Rarokar, [2]Mithali Palandurkar, [3]Dr. Renuka Mahajan

[123]Nagpur college of pharmacy, Dr Babasaheb Ambedkar Technological University, Nagpur, Maharashtra, India

ABSTRACT:

Background: Hair loss, or alopecia, is a common problem affecting both men and women. There is a growing demand for herbal cosmetics due to their minimal side effects.

Aim: The present study aims to formulate a polyherbal hair oil, designed to promote hair growth and treat alopecia. The polyherbal hair oil formulation was developed using a combination of herbs, including amla, pumpkin seed, green tea, aloe vera, almond oil, sesame oil, and coconut oil.

Method and Results: The potent chemical constituents responsible for the hair growth were determined by phytochemical screening of the herbs. Formulated polyherbal hair oil underwent a series of evaluations to ensure its quality and safety. Parameters such as viscosity, pH, saponification value, colour, odour, sensitivity, irritation, and acid value were assessed. These provided important information about the physical and chemical properties of the hair oil, ensuring its suitability for use. Increasing popularity of polyherbal hair oils for alopecia can be attributed to the growing preference for natural and safer alternatives to conventional hair care products.

Conclusion: By harnessing the therapeutic potential of herbal ingredients, these formulations offer a holistic approach to hair care, by promoting healthy hair growth. It provides a promising solution for individuals experiencing hair loss. These formulations offer the benefits of herbal ingredients with fewer side effects and improved safety and efficacy. The combination was carefully selected to obtain the best possible formulation for stimulating hair growth.

KEYWORDS: Hair loss, pH, viscosity, acid value, polyherbal

I. INTRODUCTION

Hair is one of the important parts of human body. Like other appendages of the body such as sweat glands, nails, sebaceous gland, it also protects the skin. Nowadays loss of hair is becoming a common problem in both women and men. It is because of the excessive exposure of the chemicals on the scalp, stress, thyroid, hormone, family genetics etc. Alopecia can be defined as the thinning of hair or the loss of hair or baldness and if loss of hairs occurs rapidly it can be a serious disease condition [1]

Hair is much more complicated than it seems on the surface. We all know that they not only play an important role in the appearance of men and women, but they also transmit sensory information and help create gender identity.[2] Hair consists of two main components the follicle and the visible shaft. The follicle, located within the skin, is a tunnel-like structure that extends into the dermis. It comprises multiple layers with distinct functions. At the follicle's base lies the papilla, which contains tiny blood vessels called capillaries, responsible for nourishing the cells. The bulb, surrounding the papilla and situated at the bottom of the hair, represents the living part of the hair. Remarkably, the cells in the bulb divide at a rapid rate of every 23 to 72 hours, surpassing the division speed of any other body cells. Protecting and shaping the growing hair shaft are two sheaths the inner and outer sheaths, the inner sheath extends along the hair shaft and ends just below the opening of sebaceous (oil) and occasionally apocrine (scent) glands., outer sheath remains around the gland. The hair shaft consists of three layers and is composed of a tough protein known as keratin. Interestingly, this protein is non-living, indicating that the visible hair is not a living structure.[3] The three layers include the medulla, located in the innermost part, the cortex as the middle layer, and the cuticle as the outer layer. The cortex constitutes the majority of the hair shaft, while the cuticle forms a tightly arranged structure composed of overlapping scales resembling shingles. Both the cortex and the medulla contain the pigment responsible for the hair's coloration. [4]

The follicles, situated beneath the skin's surface, are responsible for hair growth. Adjacent to the follicles, blood vessels provide essential nourishment for the follicle, contributing to hair growth. Inadequate nourishment from the blood vessels can lead to hair loss. Hair undergoes a growth cycle that encompasses five stages, starting from its initial growth phase and eventually shedding. On the scalp, hair grows approximately 0.3 to 0.4 mm per day or around 6 inches per year. Unlike other animals, human hair growth and shedding occur randomly and are not influenced by seasons or cycles. Consequently, at any given time, a varying number of hairs are in different stages of growth and shedding.

Hair Growth Cycle

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Anagen phase is a period of active hair growth in the hair follicle. During this phase, there is steady hair growth, and the cells in the follicle divide rapidly. It can last for several years and determines the maximum potential length of the hair. Catagen phase also known as transitional stage, follow the anagen phase in which the hair follicle shrinks, and hair growth ceases. It is a relatively short phase that lasts for a few weeks. Telogen phase is the resting state in which hair remains attached to the follicle without being firmly anchored. Telogen phase can last for very long time may be for several months. Exogen phase also known as the shedding phase of the hair cycle in which the old hair is naturally released from the follicle and falls out. It is a normal part of the hair cycle and allows for new hair growth to take place. New anagen phase (Kenogen phase) allows new hair growth from the same follicle, initiating a new hair cycle.

Hair loss

Most individuals typically experience the shedding of 50 to 100 hairs per day, which generally does not lead to noticeable thinning of the scalp hair due to simultaneous new hair growth. However, hair loss, baldness, or alopecia occurs when there is a disruption in the cycle of hair growth and shedding or when the hair follicle is damaged and replaced by scar tissue. While the exact cause of hair loss may not be fully comprehended, it is often associated with factors such as family history (especially for male-pattern baldness), hormonal changes (such as during pregnancy, childbirth, or menopause), certain medical conditions (including thyroid disorders, diabetes, anaemia, systemic lupus erythematosus (SLE), sarcoidosis with skin involvement, tinea scalp infection, lichen planus, and trichotillomania), specific treatments (such as chemotherapy, radiation therapy, certain medications for arthritis, depression, heart problems, high blood pressure, birth control pills, and anabolic steroids), severe emotional and physical stress, and nutritional deficiencies (such as insufficient protein or iron, eating disorders like anorexia and bulimia, and significant weight loss).[6] [7]

ALOPECIA

Alopecia is a medical term used to describe hair loss, encompassing various conditions characterized by the loss of hair. Alopecia can be caused by various factors and is not contagious, although it can sometimes be indicative of underlying health issues. One common type of alopecia is alopecia areata, which is an autoimmune disease. However, not all forms of alopecia are associated with an abnormal immune system response. Genetic, lifestyle, environmental factors, as well as psychological conditions like hair pulling, can also contribute to certain types of alopecia. Treatment approaches for different forms of alopecia may involve oral medications, topical therapies, and in some cases, behavioural changes to promote hair regrowth. Disruptions to the natural hair growth cycle can lead to alopecia, and while some forms can be prevented, others can affect individuals regardless of age, gender, race, or family history.[8] [9] The types of alopecia are androgenic alopecia, alopecia areata, cicatricial alopecia [10], persistent patchy alopecia

areata, telogen effluvium, anagen effluvium, loose anagen syndrome, trichotillomania, traction alopecia [11], postpartum alopecia, lichen planopilaris [12] and central centrifugal cicatricial alopecia.[13]

Androgenic alopecia

Androgenic alopecia, commonly known as male-pattern baldness or female-pattern baldness, is the most common form of progressive hair loss. It is influenced by a genetic and hormonal factor. The hormone dihydrotestosterone (DHT) plays a crucial role in this condition by affecting the hair follicles on the scalp. In individuals genetically predisposed to androgenetic alopecia, DHT causes a transformation in the hair follicles, resulting in the production of progressively smaller, shorter, and lighter hairs. Eventually, the affected follicles shrink completely, leading to the cessation of hair production.

Male-pattern baldness typically starts during or after puberty and affects around 50% of men by the age of 50. It follows a specific pattern, starting with a receding hairline and leading to thinning hair on the crown and temples, which may eventually result in partial or complete baldness. Female-pattern baldness primarily manifests as hair thinning on the top of the head, and it may be more noticeable in women after menopause.

It is important to note that while androgenetic alopecia is the most prevalent form of progressive hair loss, there are other types of hair loss as well. One example is alopecia areata (AA), which involves patchy hair loss and is thought to have an autoimmune component. Proper understanding and diagnosis of different types of hair loss are essential for appropriate management and treatment. [14], [15]

Alopecia areata

Alopecia areata is a common form of hair loss that typically occurs before the age of 30 in both males and females. It is characterized by sudden and patchy hair loss, predominantly affecting the scalp and beard area. The underlying cause of alopecia areata is believed to be an autoimmune disorder, in which the body's immune system erroneously targets and destroys the hair follicles. This immune response involves a combination of cell-based and humoral immunity mechanisms.

While the exact triggers for alopecia areata remain unclear, it is widely accepted that a combination of genetic, environmental, and immunological factors contribute to its development. Stress, trauma, and certain infections have been associated with triggering or worsening the condition in susceptible individuals.

Approximately 80% of patients with alopecia areata experience spontaneous hair regrowth within a year of onset. However, the extent and speed of regrowth can vary significantly among individuals. Occasionally, the regrown hair may initially appear white or gray, but it often returns to its original color over time. Treatment options for alopecia areata include topical medications, injections, and oral therapies, all of which aim to suppress the immune response and stimulate hair regrowth.[16], [17]

Telogen effluvium

Telogen effluvium (TE) is a condition characterized by a decrease in hair density and volume. This occurs as a result of a significant increase in the number of hairs shed each day and a higher proportion of hairs transitioning from the growing phase (anagen) to the shedding phase (telogen). Normally, only about 10% of scalp hair is in the telogen phase, but in telogen effluvium, this percentage rises to 30% or more. The underlying cause of TE is a disruption in the normal hair cycle.

There are various common triggers that can lead to telogen effluvium. These include childbirth, severe physical or emotional trauma, a major life event causing significant stress (such as the loss of a loved one), substantial weight loss or extreme dieting, a severe scalp-related skin problem, and the initiation or discontinuation of certain medications or hormone treatments.[18]

What Causes a Thinning, Receding Hair?

- Hair loss can have various underlying causes, with one of the most prevalent being Androgenetic Alopecia (AGA), commonly referred to as Male-Pattern Baldness (MPB).
- The primary factor attributed to Androgenetic Alopecia (AGA) is thought to be the sensitivity to dihydrotestosterone (DHT), a naturally occurring male hormone (androgen) produced by our bodies.
- Dihydrotestosterone (DHT) is derived from testosterone through the enzymatic activity of 5-alpha-reductase.
- This process occurs in different tissues of the body, including the liver and skin. A portion of the produced DHT enters the bloodstream and eventually reaches the scalp and hair follicles.[19]
- When DHT binds to androgen receptors in a susceptible hair follicle, it leads to a modification in the regular hair growth cycle of the follicle.
- spends an increased duration in the resting phase, which hinders hair growth, and a decreased duration in the active growth phase. Concurrently, the follicle gradually reduces in size. This phenomenon is commonly referred to as hair miniaturization among scientists.[20]
- The combination of shortened hair growth cycles and miniaturized follicles results in the production of increasingly shorter hair shafts. In advanced stages of baldness, the follicles eventually vanish altogether.[21]
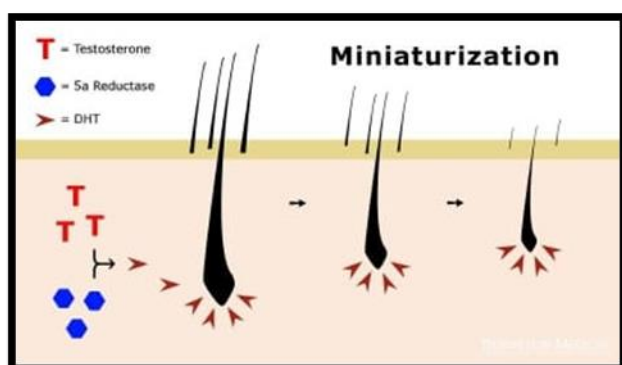

Figure 01: DHT attack on hair root

Figure 02: Miniaturization of hair due to DHT

Cause

Alopecia for male or female pattern to occur, both the gene associated with hair loss and circulating androgens must be present. The inheritance of this gene follows a multifactorial or polygenic pattern. The key initiating factor is male hormones, including androgens like testosterone and dihydrotestosterone (DHT). It has been established that DHT, which is derived from testosterone through the action of the 5alpha reductase enzyme, affects hair follicles that are genetically susceptible to its effects. This leads to hair loss through a process called miniaturization or shrinkage of the follicles. Consequently, the hair becomes progressively shorter and finer. As individuals age, genetically programmed follicles may completely cease hair growth.[22] [23]

How to Reverse Hair Miniaturization

• Remove DHT from the Scalp

• Increase Circulation and Nutrient Delivery

• Use Essential Oils

• Stop Using Chemical-Laden Hair Products

Treatment

As the above information tells us that the one of the main reasons for the hair fall (alopecia) is DHT (dihydrotestosterone) Which made up by testosterone in presence of enzyme 5-alpha- reductase. By blocking an enzyme 5-alpha- reductase, DHT formation stops and we can stop the miniaturization of hair, another method is by blocking the DHT directly also helps to stop miniaturization of hair. Various chemical based medicine currently used for blocking the action of DHT and 5-alpha- reductase enzyme such as minoxidil, finasteride, etc this medication shows some chronic side effect such as erectile dysfunction ,low sperm count and gynecomastia ,to avoid this side effect herbal product which are capable of blocking DHT and 5-alpha- reductase are used to treat hair loss , there are various herbal product which helps to improve blood circulation in scalp and nourishes the hair by using this product we can treat alopecia. One of the formulations which is mostly used for the growth of hair and which also prevents the hair loss is hair oil. By using various herbs, cosmetics can be prepared which is in great demand in the world. Addition of herbs in the cosmetics make it safe for the application to the skin. Herbal hair oil provides the natural hair growth by maintaining the moisture to the scalp and providing nutrients which is required for normal hair growth.[24] [25] [26]

Herbal ingredients and their action against alopecia

Various herbal ingredients which act against alopecia such as DHT blocker ,5 alpha reductase inhibitor and hair growth enhancer are nutritional ingredient which help naturally to reduce chances of hair loss and treat alopecia. The other herbal naturals are DHT blocker such as pumpkin seed, Saw Palmetto, 5 alpha reductase inhibitor from green tea, hair Growth enhancers from raspberry ketone, sanguisorba officinalis along with rosemary oil, witch Hazel leaf extract, geranium oil and lavender oil as Scalp improving

ingredients. Nourishing ingredients are rice protein, wheat protein, peppermint oil, aloe Vera, rosemary oil which provide nourished, healthy, and strong hair. These oils ensure a healthy scalp. Proteins helps to strengthen the hair, by repairing damaged hair, and make your hair less brittle. Biotin, Selenium, Vitamin B2 (Riboflavin), Vitamin B5 (Pantothenic Acid) provide essential nutrients to the hair.

II. PLANT PROFILE

1) Pumpkin Seed

- Plant – Cucurbita maxima
- Family – Cucurbitaceae
- Synonym – Curcubita pepo, pepita
- Purpose–Pumpkin seed act as a natural DHT blocker. [27] [28]

2) Amla

- Plant – Emblica officinalis
- Family – Euphorbiaceae
- Synonym - Myrobalan, Indian gooseberry
- Purpose – It improves its efficacy in colouring the hair & prevent premature greying, also has antioxidant property. [29] [30] [31] [32]

3) Green Tea

- Plant – Camellia sinensis
- Family – Theaceae
- Synonym – Matcha, common tea, genmaicha
- Purpose – It helps to treat baldness and restore hair volume, Certain natural products contain components that are inhibitors of 5 alpha-reductase. [33]

4) Neem

- Biological Name - Azadirachta Indica
- Family – Meliaceae
- Synonym - Melia Azadirachta. Margosa.Azadirachta indica.
- Purpose - active ingredient nimbidin. that can help suppress inflammation, which may make it useful in treating scalp irritation. [34] [35]

5) Alovera

- Biological Name - Aloe barbadensis miller
- Family – Asphodelaceae (Liliaceae)
- Synonym - Acemannan., Aloe africana., aloe gel
- Purpose - strengthens hair follicles and promotes hair growth. [36]

6) **Coconut oil**

- Biological Name - Cocos nucifera L.
- Family – Aceraceae.
- Biological source: - Oil derived from dried fruits of Cocus nucifera, kernel oil.
- Purpose - Used as vehicle, promotes hair growth. moistures the hair follicles. Stimulates hair growth by unclogging pores.

7) **Tilli oil**

- Biological Name - S. indicum.
- Family – Pedaliaceae.
- Biological source: - Seed oil.
- Purpose - It is used as a vehicle in many herbal preparations because of its stability, provides the nourishment to the dry scalp. [37] [38] [39]

8) **Almond oil**

- Biological Name - Prunus dulcis.
- Family – Rosaceae.
- Biological source: - Dried kernels of almond tree.
- Purpose - Used as vehicle, Strengthen the hair, & protect the hair from sunlight, use as scalp treatment. [40]– [42]

III. MATERIAL AND METHOD

COLLECTION OF PLANT MATERIALS

All the herbs like amla, green tea, pumpkin seed, coconut oil, till oil and almond oil purchased from Ayurvedic shop. Required solvents were collected from a laboratory.

EXTRACTION PROCESS

Amla

Alcohol Maceration: 20 g of drug is kept in a closed vessel containing 360 ml of aldol for 7 days. During the process the liquid is shaken occasionally. After 7 days the liquid is strained, and the marc is pressed. The concentrated liquid is allowed to using rotary evaporator at 50^0 C. It is then air dried for one day.

Green tea

Preparation: Begin by gathering fresh green tea leaves and ensuring they are well-cleaned and dried. Take approximately 100g of the dried leaves and grind them into a powder. Next, place the powdered green tea in a closed conical flask. Add 200ml of ethanol to the flask and allow the mixture to macerate for different

durations: 6, 12, 24, and 46 hours respectively. After the maceration process, strain the liquid from the flask and proceed to dry it using a rotary evaporator at a temperature of 50°C. Finally, leave the liquid to dry for one day.

Neem

To prepare neem extract, start by ensuring that the neem leaves are completely dried. Once dried, coarsely powder 50g of the leaves. Take 250 ml of methanol and perform successive extraction of the powdered leaves over a three-day period, shaking the mixture periodically. After extraction, filter the mixture to collect the filtrate. The filtered liquid extracts should then undergo rotary evaporation to remove solvent and concentrate the extract. This concentration step is done under reduced pressure, in a vacuum at a temperature of 40°C. Next, evaporate the extracts to dryness and store the resulting dry extract in an airtight bottle at a temperature of 4°C. [43]

Pumpkin seed

The oil extraction from pumpkin seeds was carried out using the Soxhlet procedure. Approximately 20g of crushed pumpkin seeds were placed into a lab-scale Soxhlet extractor equipped with a condenser and a 250ml round bottom flask. A food-grade solvent, such as ethanol, was added to the round bottom distillation flask, with a volume of 200ml. The heating mantle of the Soxhlet apparatus was utilized, along with a temperature controller to maintain the desired heating temperature. After heating for a specific duration, the sample's thimble, containing the extracted oil, was retrieved. The collected oil, condensed during the process, was then gathered into the flask.[44]

PHYTOCHEMICAL SCREENING OF PLANT EXTRACT

The qualitative phytochemical screening of the extract of herbs like amla, pumpkin seeds, green tea and neem was done to check the presence of active chemical constituents. The phytochemical tests are done by the following tests.

Amla

Test for tannins

Ferric chloride test: To 1ml extract ferric chloride solution is added and the formation of dark blue colour shows the presence of tannins.

Test for flavonides

Sodium hydroxide test: To the extract add large amount of sodium hydroxide solution, it shows yellow coloration which disappears after addition of acid.

Pumpkin seed

Test for proteins

Biuret test: To 3 ml extract add 40% sodium hydroxide solution and few drops of 1% copper sulphate solution. It produces blue color.

Million tests: To the test solution, million reagent is added and heated in water bath. A reddish-brown color is observed.

Green tea

Test for proteins

Biuret test: To about 3 ml of extract add 40% sodium hydroxide solution and few drops of 1% copper sulphate solution, it produces blue color.

Xanthoproteic test: To the test solution add concentrated nitric acid, boil. Yellow precipitate is formed.

Test for amino acid

Ninhydrin test: 3 ml of test solution was heated, and 3 drops of 5%Ninhydrin solution was added in boiling water and was boiled for 10 min. purple color was observed.

Neem

Test for alkaloids

Mayer's test: Few drops of Mayer's reagent is added in 3 ml of test solution cream color precipitate is observed.

Hager's test: small quantity of Hager's reagent is added in the filtrate. Formation of yellow colored precipitate is observed.

Wager's test: Few drops of Wagner's reagent is added in the solution. Formation of reddish-brown precipitate is observed.

Test for amino acids

Ninhydrin test: To the 3ml of test solution add 3 drops of 5% Ninhydrin Solution and boil for 10 min. Bluish purple color is observed.

THIN LAYER CHROMATOGRAPHY

Pre coated silica gel plates were received from the college. The plate was marked 1 cm from the bottom and spots were made with the standard and samples. Then the plate was suspended lightly in the

solvent and was allowed to run until it reaches a 3/4th position. Ninhydrin (3 % ninhydrin in 100 ml butanol containing 3 ml of Acetic acid) was used as the spraying agent and it was sprayed all over the plate and was allowed to dry. The coloured spots developed were noted and the Rf value was calculated by measuring the distance travelled by the solute and the solvent.

TLC OF AMLA

We have used different types of Mobile phase like Ethyl Acetate: Acetic Acid Glacial: Formic Acid: Water 6:1:1:2 (v/v/v/v) was used respectively [36] and Toluene: Methanol: Water in the proportion of 5:3:2 (v/v/v) was used respectively. But using Toluene: Methanol in the proportion of 8:2 (v/v) was used respectively, gives result and 3 spots where identified.

TLC OF NEEM [45]

The TLC of neem leaf extract shows the presence of triterpenoid Azadirachitin which is the active constituent of neem leaves. For TLC we have chloroform: acetone (3:1) as the mobile phase. The TLC plate was made using Silica gel G. The plate is kept in the UV chamber for visualization at 366nm.The Rf value observed is 0.37.

TLC OF PUMPKIN SEED

The TLC of pumpkin seed extract was done to check the presence of various phytochemicals in it. For TLC we used butanol: acetic acid: water in the ratio 4:1:1. The TLC plate was made using Silica gel G .The plate was kept in the UV chamber for visualization at 366 nm .The Rf value was found to be 0.19.

TLC OF GREEN TEA

The TLC of green tea was done with the mobile phase chloroform: acetone: methanol in the ratio 1:1:1. The TLC plate was made using Silica gel G .The plate was kept in UV chamber for visualization at 366 nm .The Rf value was found to be 0.53.

Formulation of hair oil

All the herbs were dried and weighed accurately. Coconut oil, almond oil, sesame oil, was taken into a vessel and heated. All the dried powder like amla, green tea, pumpkin seed and neem powder were poured into the vessel containing oil. Boiled for 20 min., stirred. Aloe vera gel was added and boiled for 10 min. with continuous stirring. After boiling cooled and strained with muslin cloth,the polyherbal hair oil is obtained.

Table 01**:** Ingredient quantity for hair oil

Sr. No.	Ingredients	Quantity
1.	Amla powder	20g
2.	Green tea powder	15g
3.	Pumpkin seed powder	40g
4.	Neem powder	10g
5.	Aloe vera gel	30ml
6.	Till oil	15ml
7.	Almond oil	40ml
8.	Coconut oil	40 ml

EVALUATION TESTS

Sensitivity test

The prepared polyherbal hair oil was applied on 1 cm skin of hand it is exposed to the sunlight 4 -5 min.

Acid Value

Preparation of 0.1 M solution: 0.56 g of KOH pellets weighed and dissolved in 100 ml of distilled water and stirred it continuously. The prepared 0.1 M KOH solution was filled in burette.

Preparation of sample: Measure 10 ml oil and dissolved in 25 ml of ethanol and 25 ml of ether and shaken well .1 ml of phenolphthalein indicator is added and titrated with 0.1 M KOH solution.

Saponification Value

Weighed accurately 1 ml of oil into 250 ml of conical flask and 10 ml ethanol: ether mixture (2:1) was added. To this flask 25 ml of 0.5 N alcoholic KOH was added. The flask was kept aside for 30 min. The cooled solution was titrated against 0.5 N HCl using phenolphthalein indicator. Similarly, the blank titration was performed without taking oil (sample). Amount of KOH in mg used was calculated.

pH measurement

The pH of prepared polyherbal hair oil was determined using pH meter.

Viscosity

The viscosity of polyherbal hair oil was determined using Ostwald's Viscometer.

Specific Gravity

Take the specific gravity bottle, rinse it with distilled water. Dry it in oven for 15 min, cool, and close it with cap weighs it (a). Now, fill the same specific gravity bottle with sample & close it with cap and again weigh it (b). Determine weight of sample per milliliter by subtracting the weight (b-a).[46] [47] [48]

IV.   RESULT

EVALUATION TABLE

The evaluation test are performed by evaluating the parameters of hair oil like color, odour, pH , acid value , saponification value , irritation test , sensitivity test . The evaluation tests are performed to check the safety and efficacy of the formulation.

Table 02: Evaluation table

Sr no .	Parameter	Formulated Preparation	Marketed Preparation
1.	Color	Brown	-
2.	Odour	Characteristics	-
3.	Acid value	3.5	2.3
4.	Saponification value	115.05	96.33
5.	pH	6.2	5-5.5
6.	Sensitivity test	No irritation	May be
7.	Irritation test	No irritation	May be
8.	Grittiness	Smooth	-
9.	Specific Gravity	0.796	0.685
10.	Viscosity	0.96 cp	0.3 cp

THIN LAYER CHROMATOGRAPHY

The separation of the crude extract by TLC using different solvent given in Table 3. TLC plate is visualised under Visible light and long UV wave(365nm).

- The spots are small & clearly defined.
- A Higher the Rf value indicates that the compound has travelled for up the plate & is less polar.
- A lower Rf value indicates that compound has not travelled far & is more polar.
- Rf = Distance travelled by compound

    Distance travelled by solvent front.

1. Amla

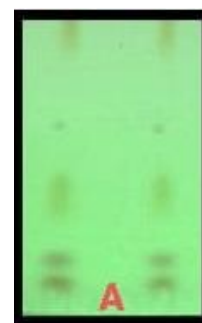   Mobile Phase: Toluene: Methanol

                          8   :   2

   Solvent Used: Small quantity of   amla powder dissolved in Ethanol.

   Standard Rf Value of Amla = **0.74 ± 0.01**

   Rf Value of = **0.81**
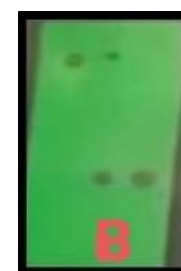

2. Neem

   Mobile phase: Chloroform: Acetone

                          3   :   1

   Solvent used: Small quantity of   extract is dissolved in Methanol

   Standard Rf Value of Neem: = **0.09 ± 0.01**

    Rf value of Neem :**0.16**

3. Pumpkin seed

   Mobile phase: Butanol : Acetic acid :Water

                          4   1   1

    Solvent used: Small quantity of   extract dissolved in ethanol .

   Rf value: **0.19**

4. Green tea

   Mobile phase :Chloroform : Acetone: Methanol

                          1   :   1   :   1

   Solvent used : Small quantity of extract dissolved in ethanol

   Rf value: **0.53**

Figure 3: TLC of (a)amla,(b)neem, (c) pumpkin seed, (d) green tea under UV

Table 03: Extractive value

Sr.no.	Ingredients	Extraction process	Yield
1.	Amla	20g of drug is taken with the whole and placed in a closed vessel containing 360 ml alcohol for 7 days with occasionally shaking.	56.25%
2.	Green tea	About 100 g of tea powder is macerated with 200 ml of ethanol in an Erlenmeyer flask for 6, 12, 24 ,46, hours respectively .	13.36%
3.	Neem	The completely dried leaves were coarsely powdered, and 50 g was used for successive extraction in 250 ml methanol for three days with periodic shaking. Then, the extract was filtrated, and the filtrate was collected. The filtered liquid extracts were subjected to rotary evaporation and subsequently concentrated under reduced pressure (in vacuum at 40°C). Then, the extracts were evaporated to dryness and stored at 4°C in an airtight bottle	16.3%
4.	Pumpkin seed	Soxhlet procedure was used to extract oils from the pumpkin seeds. About 20g of crushed pumpkin seeds were fed to lab-scale Soxhlet extractor with condenser and 250ml round bottom flask. Then, 200ml of the food grade solvent (Ethanol) was added into round bottom distillation flask. The Soxhlet apparatus was then heated up using a heating mantle with temperature controller for controlling the desire value of heating. After heating for the predetermined time, the waded thimble of sample was obtained. The condensed oil was collected into the flask.	37.58

Table 04: Phytochemical tests for Amla extract

-indicates absent and + indicates present

Phytochemical tests for amla extract			
Sr no.	**Test**	**Std result**	**Observed result**
1	Alkaloid (Hager's test)	Formation of yellow ppt	-
2	Tannin (ferric chloride solution test)	Formation of dark blue color	+
3	Saponin (foam test)	Formation of foam	-
4	Steroid (Salkowski test)	Red color in lower layer	-
5	Terpenoid (Salkowski test)	Yellow color in lower layer	-
6	Flavonoids (sodium hydroxide test)	Show yellow color which decolorize	+

Table 05: Phytochemical tests for Green tea extract

-indicates absent and + indicates present

Phytochemical tests for Green Tea extract			
Sr no.	**Test**	**Standard result**	**Result**
1	Test for Carbohydrate (Molish test)	violet ring form at junction	+
2	Test for protein (xanthoproteic test)	produce yellow ppt	+
3	Test for alkaloid (dragendroff test)	formation of orange, brown ppt	+
4	Test for saponins		+
5	Test for tannin (ferric chloride test)	dark blue color formed	+
6	Test for Flavonoids (sodium hydroxide test)	shows initially yellow color later decolorize	+
7	Test for glycoside (killer Kilani test) cardiac glycoside	formation of two layer	+
8	Terpenoid test (Salkowski test)	formation of yellow layer in lower side	+

Table 06: Phytochemical tests for Neem extract

-indicates absent and + indicates present

Phytochemical tests for Neem extract			
Sr no.	Phytochemicals (Active Constituents)	Test	Aqueous extract of neem
1	Alkaloids	Dragendroff's	+
		Wagner's	+
2	Glycosides	Benedict's	-
3	Tannins	Lead acetate	+
		Ferric chloride	+
4	Proteins	Xanthoprotein	-
		Biuret	-
5	Reducing Sugars	Benedict's	-
6	Phytosterols	Salkowski	+
7	Phenolic Compounds	FeC!3 Solution	-
8	Saponins	Foam	+

V. DISCUSSION

The formulation of a polyherbal hair oil for the treatment of alopecia has been undertaken in this study, focusing on the utilization of natural herbs known for their hair growth properties. Extensive research was conducted to identify and gather different herbs from natural sources, as they contain various phytochemicals and nutrients that promote hair growth. The process involved herb collection, extraction, phytochemical screening, and formulation evaluation. Essential constituent in herbs which blocks the DHT (Dihydrotestosterone),5 alpha reductase and by nourishing the hair providing nutrient they promote hair growth.

The plant extraction was carried out using ethanol as the solvent. Notably, amla extract yielded 56.25%, while green tea extract provided a yield of 13.36%. Phytochemical .

screening revealed the presence of tannins, flavonoids, and Vitamin C in amla extract, highlighting its therapeutic potential. Green tea extract, on the other hand, exhibited proteins and amino acids. Additionally, neem and pumpkin seeds contained proteins, alkaloids, and amino acids, further contributing to the overall efficacy of the polyherbal hair oil.

The evaluation of the polyherbal hair oil formulation encompassed various parameters to ensure its safety and effectiveness. These parameters included pH measurement, saponification value, acid value, color, odor,

and sensitivity test. The meticulous evaluation process provided valuable insights into the formulation's quality and stability, reassuring its suitability for use in treating alopecia.

VI. CONCLUSION

The formulated polyherbal hair oil shows the better hair growth activity with less or no side effects as the essential phytochemicals are present in the herbs that are required for the hair growth activity, and it also treats the different types of alopecia.

The present work of formulation has anti-dandruff, moisturizing and anti-microbial activity. The herbs used in the formulation also stimulates hair follicles and therefore helps regrowing hairs.

The polyherbal hair oil developed through this research harnesses the beneficial properties of diverse herbal ingredients and oils. Rigorous evaluation, including sensitivity tests, viscosity assessment, pH determination, saponification value analysis, acid value measurement, and other stability parameters, solidified its efficacy and safety profile. The findings of this investigation, coupled with the biological screening, establish the formulated polyherbal hair oil as an effective solution for addressing alopecia.

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