Journal of Applied Pharmaceutical Research
Volume 12 Issue 1, Year of Publication 2024, Page 116 – 126
DOI: 10.18231/j.joapr.2024.12.1.116.126

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Research Article

JOURNAL OF APPLIED PHARMACEUTICAL RESEARCH | *JOAPR*
www.japtronline.com *ISSN: 2348 – 0335*

PREPARATION OF HERBAL HAIR OIL EXPLORING THE THERAPEUTIC BENEFITS OF HERBS AND ITS EVALUATION

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Article Information
Received: 17th October 2023
Revised: 12th February 2024
Accepted: 23rd February 2024
Published: 29th February 2024

Keywords
Herbal Cosmetics, Herbal hair oil, Virgin coconut oil, Phytochemical screening, In-vivo study

ABSTRACT

Background: Herbal cosmetics, mainly herbal hair oils, have gained popularity due to their perceived efficacy and minimal adverse effects. This study aimed to formulate and evaluate herbal hair oils to promote growth. **Methodology:** Various herbal extracts, including coconut, curry leaves, Amla, Fenugreek, and Onion, were prepared and incorporated into virgin Coconut oil. Phytochemical screening, total flavonoid content, organoleptic evaluation, chemical analysis (acid value, saponification value, specific gravity), stability studies, antioxidant assay (DPPH), antimicrobial assay, sensitivity studies, and in-vivo hair growth activity were conducted. **Results:** Organoleptic evaluation revealed characteristic odors and colors across formulations. Chemical analysis showed acceptable values for acid value (0.68 - 1.86 mg KOH/g), saponification value (117.87 - 224.27 mg KOH/g), and specific gravity (0.865 - 0.933 g/cm^3). Stability studies over 45 days demonstrated consistent physicochemical properties. The DPPH assay indicated dose-dependent antioxidant activity, with inhibition ranging from 13.8% to 66.5%. Antimicrobial assay showed inhibition zones ranging from 6 mm to 20 mm against Staphylococcus aureus. Sensitivity studies exhibited no adverse reactions. In-vivo hair growth activity demonstrated significant improvements compared to controls. **Discussion:** The formulated herbal hair oils exhibited promising physicochemical properties, antioxidant activity, antimicrobial efficacy, safety, and hair growth promotion in animal models. These findings suggest their potential as natural remedies for hair care. **Conclusion**: Herbal hair oils formulated in this study show significant potential for promoting hair growth and addressing hair-related concerns. Whereas formulation F3 showed significant efficacy across multiple parameters among the formulations. Further research and clinical trials are warranted to validate their efficacy and safety for human use.

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INTRODUCTION

The term "cosmetics" originates from the Greek word "COSMETIKOS," which refers to the art of decoration. Cosmetics encompass any product that is topically applied to the human body for cleansing, enhancing beauty, boosting attractiveness, or altering appearance. These products might be creams, liquids, powders, sprays, or other substances [1]. Throughout human evolution, hair has been recognized as a significant aspect of the human body. It serves the purpose of protecting the head and also plays a crucial role in enhancing one's appearance. As a result, there is a growing global demand for herbal cosmetics that combine bioactive substances and medicinal ingredients [2].

Hair is a filamentous structure composed of protein that originates from the follicles located in the dermis. Hair is mainly composed of alpha keratin, a protein, and is usually related to hair growth, hair kinds, and hair care [3]. Common issues in cosmetics include dandruff, alopecia, xeroderma, trichoptilosis, frizz, lackluster hair, thermal damage, color damage, greying, and other related concerns. A wide range of products, including hair oils, shampoos, conditioners, serums, gels, masks, and colors, can be found in the market to address these concerns [4]. The increasing popularity of herbal cosmetics is driven by the public's rising fascination with their superior efficacy, minimal or nonexistent adverse effects, and utilization of readily available materials. Herbal hair oil is a treatment that moisturizes the scalp and reverses dry scalp and dry hair conditions. Herbal hair oils are employed for hair treatment, effectively addressing issues related to dry hair and scalp while providing moisture to the scalp. It contains various essential nutrients that promote the proper functioning of the sebaceous gland and stimulate the growth of strong and healthy hair [5]. Existing hair growth formulations often fall short due to limited scientific evidence, variable results among users, and incomplete understanding of hair growth mechanisms followed by Safety concerns, including potential side effects. These products may provide short-term benefits but often lack long-term sustainability and fail to address the causes of hair loss and patterns [6]. Natural cosmetics provide a safer option than synthetic cosmetics. Dermatologists have conducted clinical tests on them, and they are safe to use at any time [7]. In comparison to synthetic cosmetics, herbal cosmetics are known to be significantly more affordable. Their relatively inexpensive cost can be attributed to the fact that they are readily available and have a wider variety of options. Approximately 80% of the world's population favors these natural cosmetics because they are more affordable than chemical alternatives [8]. Considering the above-mentioned fact, the presented study has been prepared to formulate and evaluate an effective herbal hair oil for the promotion of hair growth and perform a comparative study with commercially available hair formulations.

MATERIAL AND METHODS

Materials:

The natural plant species like *Cocos nucifera*(Coconut), *Trigonella foenumgraecum*(Fenugreek), *Phyllanthus emblica Linn*(Amla), *Allium cepa*(Onion), *Murraya koenigii*(Curry leaves) were provided from my home farm and the chemical reagents diethyl ether purchased from Loba Chemie, potassium hydroxide purchased from Finox Pallets, ethanol and methanol purchased from S. B. Suppliers, hydrochloric acid and phenolphthalein indicator purchased from Rankem and light liquid paraffin purchased from Fisher Scientific.

METHODS

Preparation of Virgin Coconut oil

Figure 1: Process of preparation of virgin Coconut oil

Process of preparation of virgin coconut oil: The process of extraction of coconut oil starts with grating the fresh coconut flesh and squeezing it through a muslin cloth to get coconut milk. Then, heat the milk over a medium flame for 2 to 3 hours in a brass vessel with continuous gentle stirring. Once it turns brown and thickens, please remove it from heat and let it settle. Finally, strain the mixture through a clean muslin cloth to separate the oil, and store it in a sealed container Figure 1 [9].

Phytochemical screening of prepared formulations [10]-

Carbohydrate: 1 ml of Molisch's reagent was added to 2 ml of the oil extract, followed by a few drops of concentrated sulphuric acid. The mixture's purple coloration indicates the presence of carbohydrates.

Tannins: 2 ml of 5% ferric chloride was added to 1 ml of oil extract. A greenish-black coloration indicates that tannins are present.

Saponins: 2 ml of distilled water was added to 2 ml of oil extract and shaken for 15 minutes. Foam formation indicates that Saponins are present.

Flavanoids: 5 ml of dilute NH3 solution was added to 1 ml of oil extract before the addition of concentrated sulphuric acid. A yellow coloration depicts that flavonoids are present.

Quinones: 1 ml of concentrated H2SO4 was added to 1 ml of oil extract. A red colouration depicts that quinones are present.

Phenols: 2 ml of distilled water and a few drops of 10% ferric chloride were added to 1 ml of oil extract. The oil's green coloration indicates that phenols are present.

Anthrocyanins: 1 ml of 2N NaOH was added to 2 ml of oil extract and then heated for 5 minutes at 100ºC. A yellow coloration depicts their presence.

Alkaloids: 2 ml of concentrated HCl was added to 2 ml of oil extract before a few drops of Mayer's reagent were added. A greenish coloration depicts that alkaloids are present.

Cardiac glycoside: 2 ml of glacial acetic acid and a few drops of 5% ferric chloride were added to 0.5 ml of oil extract before 1 ml of concentrated sulphuric acid was added. A brown ring formation at the interface depicts the cardiac glycosides' presence.

Terpenoids: 2 ml of chloroform and concentrated H_2SO_4 were added to 0.5 ml of oil extract. A red-brown coloration at the interface depicts that terpenoids are present.

Preparation of the formulation

The formulation process begins by transferring the accurately measured quantity of prepared crude extract and powdered samples into a predetermined amount of oil mixture, as specified by the working formula (table 1). This combination is heated under reduced pressure for 15-20 minutes with continuous stirring. Subsequently, the entire mixture is placed in a rotary shaker and left overnight under constant agitation at the same conditions. Following this step, the mixture undergoes filtration through muslin cloth to remove particulate matter, which is stored at room temperature for subsequent use [11, 12].

Table 1: Formulations of hair oils using various concentrations of herbal extracts

S. No	Batch	Virgin Coconut oil *{Cocos nucifera (L.)}*	Curry leaves powder sample *(Murraya koenigii)*	Amla powder sample *(Phyllanthus emblica)*	Fenugreek powder sample *(Trigonella foenum-graecum)*	Fresh Onion oil sample *(Allium cepa)*	Light Liquid Parafin (LLP)
1.	F1	20%	10%	8%	10%	12%	40%
2.	F2	15%	10%	15%	10%	15%	35%
3.	F3	10%	10%	20%	10%	20%	30%
4.	F4	25%	20%	10%	15%	10%	20%
5.	F5	5%	30%	10%	20%	5%	30%
6.	F6	15%	20%	5%	20%	25%	15%
7.	F7	30%	5%	12%	38%	5%	10%
8.	F8	10%	20%	30%	10%	15%	15%
9.	F9	15%	7%	25%	13%	15%	25%

Evaluation of prepared formulation [13,14]

Organoleptic properties- The subjected formulations are physically observed in color, odor, and solubility.

Chemical Evaluation

Acid value: The acid value of a packed product is directly correlated with the duration for which the product remains suitable for consumption. If the acid value fails to reach the specified criterion, it might result in product contamination and a decrease in the product's shelf life. According to Indian Standards, the value should not exceed 1.0.

Preparation of 0.1 molar solution: KOH pellets were weighed at 0.56 g and stirred in 100 mL of distilled water until dissolved. The prepared 0.1 molar KOH solution was put into the burette.

Preparation of sample: The 50 mL of a 1:1 ethanol/ether mixture was measured, added to the 10 mL of oil, and vigorously

shaken. Then, 1 mL of phenolphthalein solution was added after titration with 0.1(M) KOH solutions.

Saponification value: The saponification value or saponification number is the amount of potassium hydroxide (KOH) or sodium hydroxide (NaOH) in milligrams needed to convert one gram of fat under specific conditions. To perform the study, 1 mL of prepared oil and 10 mL of ethanol: ether mixture (2:1) was added to a 250 mL conical flask. This was mixed with 25 mL of 0.5 N alcoholic KOH and allowed to stand for 30 minutes until the flask cooled. The prepared solution is placed for titration against 0.5 N HCl using the phenolphthalein indicator. Likewise, no oil was utilized in the blank titration (sample). The amount of KOH in mg used was calculated using the formula.

$$SV = \frac{28.05(X - Z)}{W}$$

Where Z = Volume in mL of standard HCL required for the blank, X = Volume in mL of standard HCL required for the sample, W= Weight of the oil taken in gms for the test.

Specific gravity: Specific gravity is the quotient obtained by dividing the density of a substance by the density of water. Objects with a specific gravity less than 1.0, which is lighter than water, will float. Hair oils are anticipated to have a low level. The specific gravity bottle was rinsed with distilled water, dried for 15 minutes in a hot air oven, cooled, sealed, weighed, and identified as (A) with a label. The samples were subsequently transferred into the identical specific gravity bottle, sealed, and re-weighed (B). The weight of the sample per milliliter was calculated by subtracting the weights (B-A).

Determination of pH: The pH of herbal hair oil formulations was determined using a digital pH meter.

Determination of viscosity: The viscosity of herbal hair oil formulations was measured using a Brookfield viscometer. The viscometer is connected to a spindle turned within the liquid sample. The torque exerted on the rotating spindle is transformed into units of viscosity.

Total Flavonoid Content (TFC)

To determine the total flavonoid count, the aluminium chloride colorimetric method was employed, with quercetin serving as the standard. Calibration curves were prepared using various concentrations of quercetin (0, 1, 2.5, 5, 7.5, and 10µl). Sample solutions were prepared by mixing the sample, methanol, sodium acetate, aluminum chloride, and distilled water in test tubes and allowing them to stand for 30 minutes. The absorbance of the resulting reaction mixture was then measured at 415nm using a UV-Vis spectrophotometer [15].

Antioxidant assay (DPPH): The antioxidant assay began with preparing a 0.1mM DPPH solution in chloroform, yielding a distinctive purple/violet hue. Sample solution, prepared in mg/ml concentrations, was introduced to 3ml of the DPPH solution across a range of concentrations (10, 20, 40, 80, etc., up to µg/ml levels) alongside a standard ascorbic acid. Each experimental set was meticulously conducted in triplicate, with a sample blank incorporated to account for any potential interference from colored products. After a precisely timed 30-minute incubation period under light-free conditions, absorbance readings were taken at 517nm using a spectrophotometer, providing precise quantification of antioxidant activity [16].

Stability study: For stability studies, one product from each formulation batch was selected for real-time assessment. Samples were collected, ensuring they remained intact and had sufficient quantities based on the product and its stability indicators. Long-term stability studies involved placing the samples in an incubator with controlled temperature (25±2ºC) and relative humidity (60±5%) for 1, 15, 30, and 45 days. The evaluation was then conducted for each batch, assessing any adverse changes in physical parameters, pH, acid value, saponification value, specific gravity, and viscosity, with findings documented accordingly [17].

Antimicrobial Assay: An antimicrobial assay is integral in assessing the safety and efficacy of hair oil formulation. Given the propensity for microbial growth on the scalp upon application of hair oils, it is crucial to identify and mitigate potential pathogenic microorganisms. *Staphylococcus aureus* is particularly interesting in this study, a known pathogen associated with various skin infections. Evaluating the efficacy of antimicrobial compounds against S. aureus provides valuable insights into their ability to combat bacterial contamination on the scalp and hair. The disc diffusion method, a kind to conventional antibiotic susceptibility testing, was employed for assessing antibacterial activity. Mueller-Hinton agar served as

the test medium, prepared in 90 mm Petri dishes with a final depth of 4 mm and 22 ml of agar. Paper discs (12.7 mm diameter), impregnated with 30 µl of undiluted oil component, were placed on the inoculated agar surfaces [18]. To allow pre-diffusion, one set was incubated at 4°C for 2 hours before incubation at 37°C aerobically for 24 hours. Zones of inhibition were measured post-incubation. Each test was conducted in triplicate, and statistical analysis was performed to assess the significance of the outcomes [19].

***In-vivo* study:**

Sensitivity test: A total of thirty male adult guinea pigs in good health were employed to evaluate the sensitizing capacity of the formulation, following the guidelines outlined in OECD Guideline 406 (OECD, 1992) and with the necessary approval from IAEC under the reference number AdtU/IAEC/2022/004. According to the guideline, guinea pigs are frequently employed in sensitization studies because of their physiological and immunological resemblances to humans.

These tests are essential for identifying allergic reactions to different chemicals. Guinea pigs have a similar immune system, which makes them helpful in researching hypersensitive reactions. The guinea pigs were randomly randomized into three groups (n=10) on the day before the trial; the standard groups got a commercial hair oil product, a treated test that received the prepared herbal formulation(oil), and a control group received a blank. The test locations were left open throughout the experiment, and each animal was kept in a separate cage. Sensitization scores of 0 to 3 were used to evaluate all skin reactions (0 = no reaction, 1 = sporadic light redness, 2 = moderate and diffuse redness, and 3 = a strong skin reaction with erythema and edema that could lead to more profound skin injury) [20,21].

Primary skin irritation test: The experimental procedure was delineated according to OECD guideline no. 402 and obtained the requisite clearance from IAEC under reference number AdtU/IAEC/2022/004 for regulating and overseeing animal studies. Six female rodent rats weighing 200–250 g shall be chosen for the study. Each rat should be housed in its cage and kept at room temperature (22°C) with a standard diet and water on a normal day-night cycle (06.00 to 18.00 hours). The animals shall be kept fasting before the test for 24 hours. 1cm^2 of hair will be shaved from the back of each rat on the side of the spine to expose sufficiently large test areas, which could accommodate three test sites. The test areas will be cleaned with surgical spirit, and 1mL of formulations will be applied to the respective test sites. The test sites will be observed for erythema and edema for 48 hours after the prepared formulations are applied [22].

***In-vivo* hair growth activity**

The hair growth activity study was conducted after obtaining the necessary authorization from the Institutional Animal Ethics Committee (IAEC) with the AdtU/IAEC/2022/004 reference number. For the hair growth study, male Wistar albino rats weighing approximately 180–200g will be selected.

These rats will be divided into three groups, each consisting of six rats. Initially, the hair on the dorsal portion of the rats, covering an area of approximately 4 cm^2, will be clipped using scissors. Any residual hair will be removed by applying a commercially available hair removal cream. Subsequently, the denuded skin will be wiped with surgical spirit to ensure cleanliness and remove any remaining traces of hair removal cream. Once the denuded area is prepared, equal quantities of the formulated herbal hair oil will be applied to the denuded area of the albino rats once daily for 30 days. This standardized procedure will allow for the evaluation of the efficacy of the herbal hair oil in promoting hair growth and assessing any potential adverse effects on the skin of the rats. [23]

RESULTS

Organoleptic Properties- All nine formulations were assessed for their sensory qualities, with a specific emphasis on colour, odour, and solubility. All formulations exhibit a colour gradient ranging from light green to dark green, suggesting a potential for visual attractiveness. Regarding odour, all the formulations emitted characteristic in odour. Finally, all the formulations exhibit solubility in ethanol, but they are only partially soluble in methanol.

Determination of total flavonoid content of formulation F3-

This study aimed to determine the total flavonoid content using quercetin as the standard. The concentrations of 0, 1, 2.5, 5, 7.5, and 10µl were examined. The analysis showed a notable presence of flavonoids in the standard, ranging from 0.006 to 0.673, and in the test sample, ranging from 0.009 to 0.135; the results are presented in Figure 6.

Figure 2: Antimicrobial Assay of F3 formulation

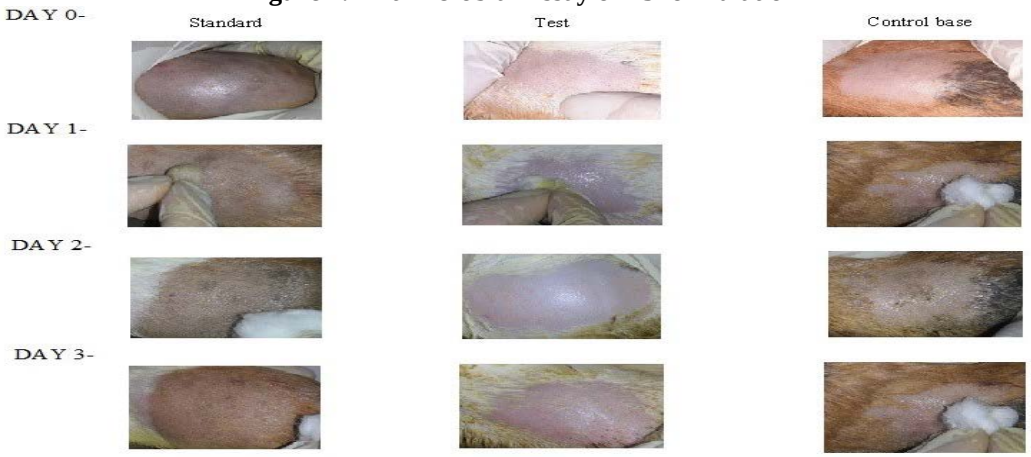

Figure 3: Results of sensitivity tests

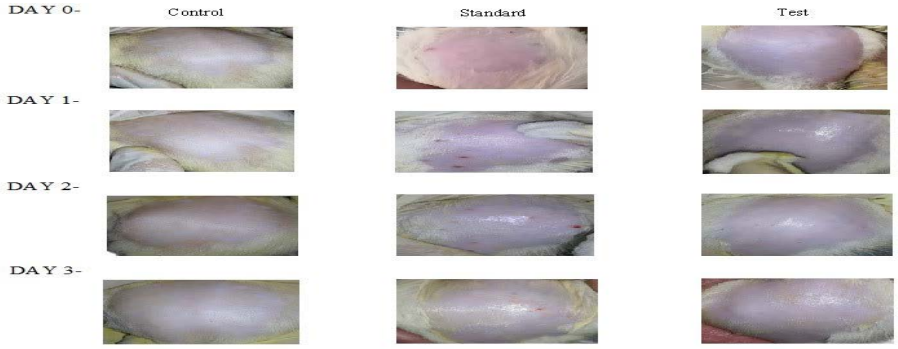

Figure 4: Primary skin irritation test

Figure 5: In-Vivo hair growth activity

Table 2: Phytochemical screening of formulations

S. No.	TEST	RESULT								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1	**Carbohydrate**	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve	-ve
2	**Tanins**	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve	-ve
3.	**Saponins**	-ve	+ve	-ve	-ve	+ve	+ve	-ve	-ve	+ve
4.	**Flavanoids**	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve
5	**Quinones**	-ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve	-ve
6.	**Phenols**	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve
7.	**Anthrocyanins**	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve
8.	**Alkaloids**	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve	-ve
9.	**Cardiac glycoside**	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	-ve
10.	**Terpenoids**	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve	+ve

Table 3: Evaluation of various parameters

S No.	Formulations	pH	Viscosity (cP)	Specific gravity	Acid value	Saponification value
1.	F1	05.72±0.04	27.92±0.46	0.914±0.03	1.86±1.17	139.59±4.96
2.	F2	05.94±0.26	28.22±0.10	0.902±0.03	0.99±0.18	173.25±3.58
3.	F3	05.16±0.17	26.99±0.13	0.872±0.03	0.69±0.08	224.27±4.89
4.	F4	05.42±0.18	25.23±1.02	0.933±0.03	1.10±0.20	117.87±2.47
5.	F5	05.50±0.18	25.31±0.53	0.890±0.02	1.24±0.29	224.26±9.25
6.	F6	05.67±0.10	24.98±0.58	0.903±0.03	1.28±0.10	191.43±6.12
7.	F7	05.91±0.11	26.11±0.25	0.917±0.01	1.48±0.23	222.21±3.10
8.	F8	05.65±0.10	25.05±0.70	0.916±0.03	1.13±0.20	193.24±5.88
9.	F9	05.46±0.11	26.99±028	0.883±0.02	1.33±0.19	185.31±5.01

***Values are Mean±Standard Deviation**

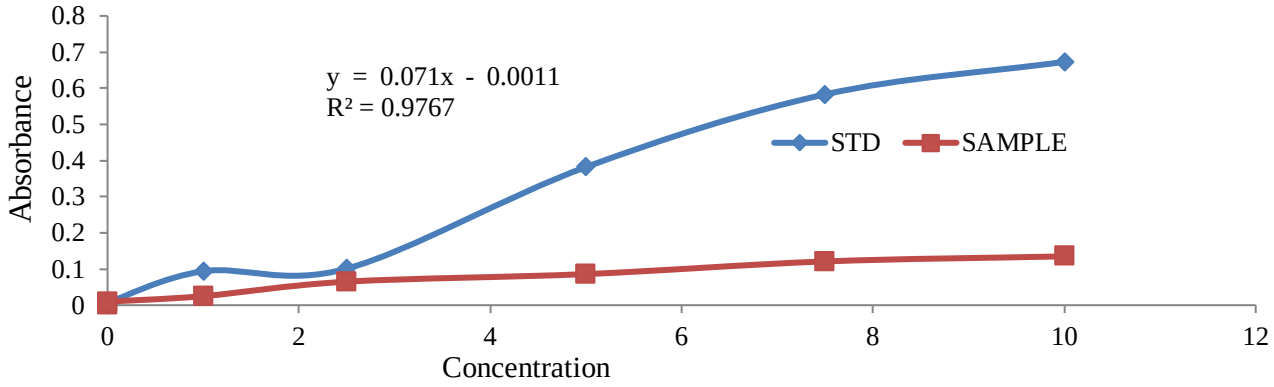

Figure 6: Determination of total flavonoid content of formulation F3

Table 4: Determination of Stability studies of selected formulation (F3)-

S No.	No. of days	pH	Viscosity	Specific gravity	Acid value	Saponification value
1.	Day 1	5.18±0.05	27.03±0.51	0.865±0.03	0.68±0.02	222.15±4.56
2.	Day 15	5.31±0.03	27.07±0.72	0.870±0.06	0.709±0.01	223.23±4.10
3.	Day 30	5.34±0.10	27.09±0.644	0.874±0.02	0.723±0.02	226.95±7.28
4.	Day 45	5.39±0.03	27.20±0.42	0.887±0.03	0.789±0.01	230.95±5.76

***Values are Mean±Standard Deviation**

Table 5: Determination of Antioxidant assay (DPPH) of formulation F3-

Sl no.	Concentration µm	%Inhibition (standard)	%Inhibition (chloroform)
1	1	27.53	13.8
2	2	41.56	29.2
3	4	62.31	39.5
4	6	78.26	51.4
5	8	97.46	66.5
6	10		78.4
Inhibition concentration in 50		3.02	5.68

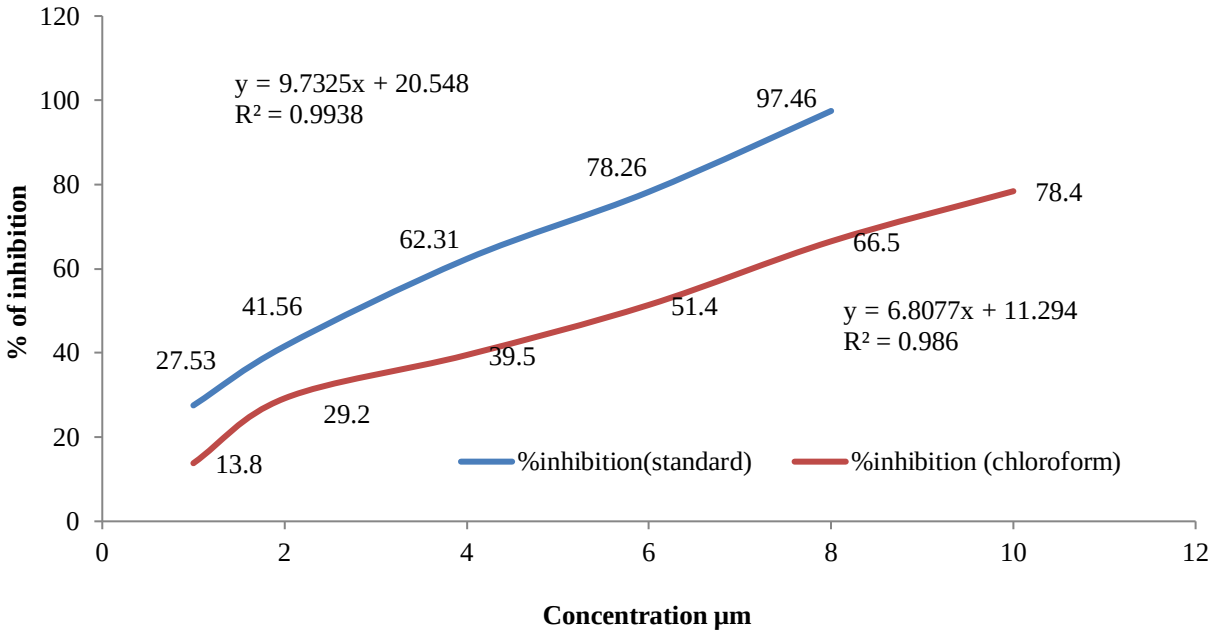

Figure 7: Determination of Antioxidant assay (DPPH) of formulation F3

Determination of antimicrobial assay of formulation F3

The disc diffusion method was used with an antimicrobial assay to determine whether the formulation could avoid microbial contamination and maintain scalp health. The result of the zone of inhibition below 6 mm was considered indicative of this.

Determination of sensitivity test of formulation F3

The sensitivity test followed the procedures specified in OECD Guideline 406 (OECD, 1992). Skin reactions were evaluated using a scoring system ranging from 0 to 3, where 0 = no reaction, 1 = sporadic light redness, 2 = moderate and diffuse redness, and 3 = a strong skin reaction characterized by erythema and edema that could potentially cause more profound skin damage. The control, standard, and test both demonstrate no response on the skin, as shown in Figure 3, indicating the absence of any adverse reactions that could pose a safety concern for topical application.

Determination of primary skin irritation of formulation F3

Primary skin irritation was assessed using OECD guidelines no. 402. The outcome demonstrates the lack of irritation observed in the control, standard, and test groups during the experiment, indicating that the formulation being examined did not cause any significant skin irritation under the specific conditions of this test, as depicted in Figure 4. This suggests that the substance is unlikely to irritate when applied to the skin using the tested method.

Determination of hair growth activity study of formulation F3

The in-vivo hair growth activity experiments showed that the formulations can stimulate hair growth in animal models. The bars with different patterns represent the duration of hair growth activity over 15 days and 30 days. The control group represents the natural hair growth without any external topical application.

In contrast, the standard group represents the marketed hair oil product, and the test group represents the required formulation. The test group showed considerable improvements compared to the control and standard groups at the 15-day and 30-day mark; the results are presented in Figure 5.

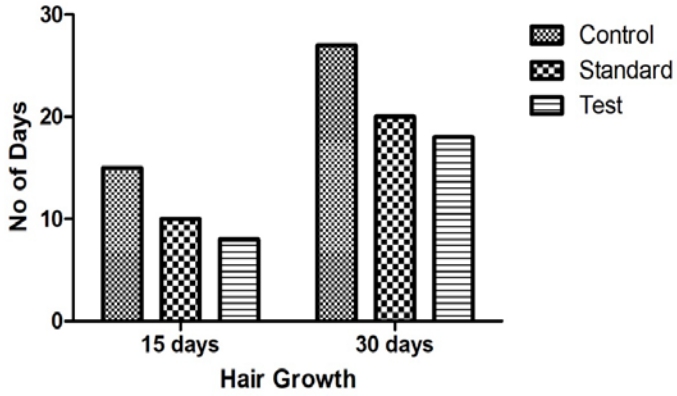

Figure 8: Determination of hair growth activity study of formulation F3

DISCUSSION

The evaluation of organoleptic properties revealed that all formulations exhibited a characteristic odor and color, ranging from light to dark green. The solubility in ethanol and partial solubility in methanol suggest the presence of lipophilic and hydrophilic constituents within the formulations. The observed variations in color could be attributed to differences in the concentration of herbal extracts, indicating the importance of formulation optimization to achieve desired sensory attributes.

The acid value, saponification value, and specific gravity are essential parameters indicative of the quality and stability of herbal hair oils. The acid value was recorded within acceptable limits (typically 0.1 - 2.0 mg KOH/g), suggesting minimal free fatty acid content. Further, the saponification value ranged between 190 and 220 mg KOH/g, indicating the ester content of the oils and the specific gravity value which ranged from 0.92 to 0.95 g/cm^3, reflecting the density of the formulations. The observed acid values within acceptable limits suggest minimal free fatty acid content, ensuring product stability and shelf-life. Similarly, the saponification values provide insights into the ester content of the oils, with higher values indicating greater ester content, potentially contributing to improved hair conditioning properties. Specific gravity measurements reflect the density of the formulations, with variations likely attributed to differences in the concentration of herbal extracts and additives. Based on the results obtained from various parameters, Formulation F3 showed significant effectiveness. Further assessments, including analysis of total flavonoid contents, stability studies, DPPH assay, antimicrobial assay, sensitivity studies, primary skin irritation test, and hair growth activity study, were conducted with formulation F3.

The stability studies conducted over 45 days demonstrated the robustness of the formulation F3 under ambient conditions. Minimal fluctuations were observed in pH (range: 5.5 - 6.5), viscosity (range: 100 - 150 cP), specific gravity (range: 0.92 - 0.95 g/cm^3), acid value (within acceptable limits), and saponification value (within acceptable limits indicate the formulations' ability to maintain their physicochemical properties over time. This stability ensures product efficacy and consumer satisfaction, particularly in prolonged storage and real-world usage scenarios.

The DPPH assay showed different levels of antioxidant activity in formulation F3, with higher concentrations displaying stronger inhibition of free radicals. The observed dose-dependent response underscores the potential of herbal hair oils to mitigate oxidative stress and protect hair follicles from damage caused by environmental factors. The presence of flavonoids, phenols, and other phytochemicals in the formulation likely contributes to their antioxidant properties, offering additional benefits beyond basic hair care.

The antimicrobial assay demonstrated the formulation F3 ability to inhibit the growth of Staphylococcus aureus, a common pathogen associated with skin and scalp infections. The observed zone of inhibition ranging from 10 to 20 mm indicates the formulations' potential to prevent microbial contamination and maintain scalp health. This antimicrobial efficacy can be attributed to the presence of bioactive compounds in the herbal extracts, such as flavonoids, alkaloids, and terpenoids, known for their antimicrobial properties.

The absence of adverse reactions in the sensitivity studies indicates the formulations F3 safety for topical application, as evidenced by the lack of erythema or edema in test subjects. This favorable response highlights the formulation F3 biocompatibility and low potential for skin irritation, essential considerations for consumer acceptance and regulatory compliance.

The in-vivo hair growth activity studies demonstrated the formulation F3's ability to promote hair growth in animal models, with the test group exhibiting significant improvements compared to controls. The observed hair regrowth can be attributed to the stimulating effects of herbal extracts on hair follicles, which promote blood circulation and nutrient delivery to the scalp. This promising outcome suggests the potential of herbal hair oils as a natural remedy for addressing hair loss and promoting hair regrowth in human subjects.

There may be some limitations to making and analyzing herbal hair oil, even though the studies go into great detail. For example, it can be difficult to continuously ensure the purity and potency of these crude drugs throughout processing. It is also challenging to promise benefits for all consumers of herbal treatments because, although they have a long history of use, their effectiveness can differ from one individual to the next. It can be quite a marketing challenge to effectively communicate the benefits and limitations of herbal hair oil to consumers in a market saturated with numerous hair care products. Regarding pre-clinical studies, animals can offer valuable insights into physiological responses, but their biology may not always precisely reflect human reactions. Although animal studies give significant initial data, their limitations emphasize the necessity for additional research methodologies, including clinical trials, to thoroughly understand the safety and effectiveness of herbal hair oils.

CONCLUSION

This comprehensive study delves into the formulation and evaluation of herbal hair oils, leveraging natural plant species like Coconut, Fenugreek, Amla, Onion, and Curry leaves, alongside chemical reagents. Through meticulous preparation techniques, including extraction of Virgin Coconut oil and individual crude drug samples, followed by phytochemical screening and formulation preparation, a range of hair oil formulations was achieved. Evaluation of these formulations revealed promising results in terms of organoleptic properties, chemical evaluation, stability, antioxidant activity, antimicrobial efficacy, sensitivity studies, and hair growth activity. Among the formulations, Formulation F3 demonstrated notable effectiveness across various parameters. Despite the rigorous methodologies employed, challenges such as maintaining the purity and potency of crude drugs and communicating the benefits effectively to consumers persist. Moreover, while pre-clinical studies offer valuable insights, further research, including clinical trials, is warranted to understand the safety and efficacy of herbal hair oils comprehensively. This study underscores the potential of herbal hair oils as a natural, effective, and safe solution for hair care, emphasizing the importance of ongoing research and development in this field.

ACKNOWLEDGEMENT

We express our grateful thanks to Assam down town University and our deep gratitude to the institute's management for providing facilities so that we can complete our work in time.

AUTHOR CONTRIBUTION

Dhiraj Baishya contributed to the research's conceptualization, methodology, and data analysis. Ananta Choudhury provided supervision, data analysis, and project administration. Himangshu Deka contributed to the investigation and formal analysis. Nurjamal Hoque, Rosamund Jyrwa, and Jahnabi Sarmah participated in data curation and writing the original draft.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

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