

# Design and Evaluation of Hair Growth - Hair Fall Oil Formulation from Botanicals

Md. Jahid Hossain<sup>1</sup>, Omar Faruk<sup>2</sup>, Dilshad Noor Lira<sup>1</sup>, Diponkor Kumar Shill<sup>3</sup>  
and Abu Shara Shamsur Rouf<sup>1</sup>

<sup>1</sup>Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka  
Dhaka-1000, Bangladesh

<sup>2</sup>Department of Pharmacy, Faculty of Sciences and Engineering, East West University, Dhaka-1212, Bangladesh

<sup>3</sup>Department of Pharmacy, Faculty of Life and Earth Sciences, Jagannath University, Dhaka-1100, Bangladesh

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## Abstract

Hair loss in adult men albeit common, can cause emotional distress. Many people with hair fall issues try chemical-based treatments that may lead to health complications and allergic reactions. Cosmetics using natural ingredients derived from plants have fewer side effects compared to synthetic alternatives. The study aimed to formulate herbal hair oil using various ingredients like castor oil, sunflower oil, jojoba oil, olive oil, pumpkin oil, etc. and evaluate its effectiveness in promoting hair growth and prevention of hair fall (HG-HF). Besides, the oil was supposed to provide a range of benefits for hair health, including preventing dandruff, improving scalp health, providing antioxidant properties, and thereby protecting against oxidative damage. The DPPH radical scavenging test was done to evaluate the antioxidant activity of the oils. Varying IC<sub>50</sub> values were found such as olive oil and sunflower oil showed IC<sub>50</sub> values of 3.79 µg/ml and 4.44 µg/ml respectively. Hence, these oils have significant antioxidant activity with a strong ability to scavenge or neutralize free radicals. A study was conducted on sixteen volunteers with hair loss, dandruff, and other hair problems to check the performance of the oil. 56.25% of the participants experienced increased hair growth and 50% reported a decrease in hair fall after using the oil for 3 months, with no remarkable side effects. Data demonstrated effectiveness and overall acceptable perception of the HG-HF oil by the participants.

**Key words:** Hair loss, hair growth, herbal, IC<sub>50</sub>, antioxidant, oxidative damage.

## Introduction

Hair plays a significant role in self-image, social perceptions and psychological well-being. It holds great importance in shaping our lives and can significantly influence our confidence and sense of identity (Alfonso *et al.*, 2005). The average scalp contains approximately 100,000 hairs, with over 90% of them actively growing in a phase called anagen (Majeed *et al.*, 2020). Hair continuously cycles and regenerates on the scalp, with each hair shaft typically lasting 2 to 6 years before falling out and being replaced by new hair (Figure 1) (Sm *et al.*, 2010).

After the anagen phase, there is a 3-week period known as catagen, during which programmed cell death occurs (Majeed *et al.*, 2020). Following catagen, the hair enters a resting phase called telogen, which lasts for about 3 months (Williamson and Gonzalez, 2001). Compared to anagen hairs, telogen hairs are positioned higher in the skin and can be easily pulled out. Normally, around 100 telogen hairs are shed from the scalp daily, which is considered a normal occurrence (Abelan *et al.*, 2022; Shapiro, 2007).

**Corresponding author:** Abu Shara Shamsur Rouf; Email: rouf321@yahoo.com; Mobile: +8801916670403

**Corresponding author:** Dilshad Noor Lira; Email: dlira.1125@du.ac.bd; Mobile: +8801719072305

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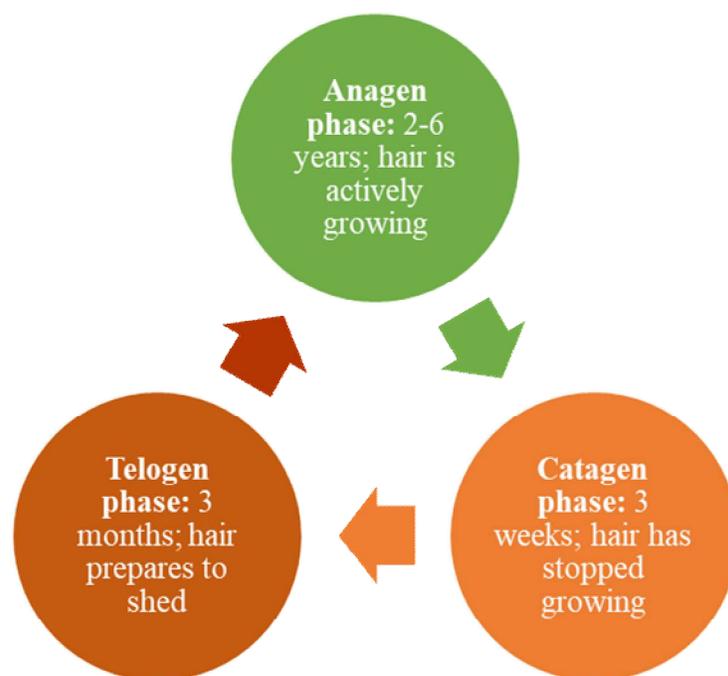


Figure 1. Normal hair life cycle (Dhariwala and Ravikumar, 2019).

Alopecia, a dermatological disorder characterized by the partial or complete absence of hair, is a common concern in both cosmetic and primary healthcare practices (PK and DJ, 2015). Alopecia affects a significant portion of the global population, estimated to be between 0.2% and 2% (Patel *et al.*, 2015). It is a prevalent issue that affects both men and women, and it can occur at different stages of life, including adulthood and after puberty. It can be attributed to various factors, including genetics, hormonal changes, nutritional deficiencies, medical conditions, certain medications, stress, and hairstyling practices (PK and DJ, 2015). One of the key reasons for alopecia is the hormone dihydrotestosterone (DHT) which is a potent hormone derived from testosterone by the enzyme 5-alpha-reductase (Patel *et al.*, 2015). DHT binds more easily and remains bound for longer periods compared to testosterone. Excess DHT is associated with hair follicle miniaturization, leading to a decrease in the growth phase and an increase in the resting phase of hair, ultimately causing alopecia (Dhariwala and Ravikumar, 2019). DHT-related hair loss, known as

male pattern hair loss or androgenetic alopecia (AGA), is more common in men (Patel *et al.*, 2015). Factors like UV radiation, humidity, wind and chemicals in hair products can damage the hair, affecting its growth and texture (Monselise *et al.*, 2015). Grooming habits and weathering together can worsen age-related hair thinning. To maintain healthy hair, it is important to protect it from environmental factors, avoid excessive heat styling and seek professional advice for personalized care (Sadick and Arruda, 2021).

There are several treatment options available for hair loss. It is important to note that the suitability and effectiveness of these treatments can vary depending on the underlying cause of hair loss, individual factors and the advice of a healthcare professional. Minoxidil, a topical medication applied directly to the scalp, is available over the counter and can help promote hair growth and slow down hair loss in both men and women (Gupta *et al.*, 2021). It is typically used for androgenic alopecia (AGA) and can be effective in certain cases (Rossi *et al.*, 2012). Finasteride is an oral medication that works by

inhibiting the enzyme 5-alpha-reductase, which converts testosterone to DHT (Ross and Shapiro, 2005). It is primarily used to treat AGA in men. It can help slow down hair loss and in some cases promote regrowth (Cho *et al.*, 2014). Glucocorticoids are anti-inflammatory medications that are often used to treat alopecia areata, an autoimmune disorder that causes patchy hair loss (Rice, 1999). Anthralin is another topical medication used to treat conditions like alopecia areata and psoriasis which works by reducing inflammation and regulating the immune response in the scalp (Rice, 1999). Spironolactone is an oral medication that is primarily used to treat high blood pressure but can also be prescribed off-label for certain types of hair loss, particularly in women. It helps block the effects of androgens on the hair follicles (Rice, 1999). There are also surgical procedures including hair transplantation which involves taking healthy hair follicles from one area of the scalp (typically the back or sides) and transplanting them to areas with thinning or no hair (Shapiro, 2007). However, these medications are associated with high costs and various undesirable side effects, including erectile dysfunction, abnormal ejaculation, reduced ejaculatory volume, sexual dysfunction, breast enlargement (gynecomastia), testicular pain, impaired muscle growth and severe myopathy (Lourith and Kanlayavattanakul, 2013; Rice, 1999). On such occasions, botanicals-derived hair-care products can offer long-term effective solutions with less or no irritation (Abelan *et al.*, 2022).

The increasing demand for natural cosmetics has led to a growing interest in essential oils. These oils, derived from natural sources, contain biologically active compounds with diverse chemical compositions, making them valuable for various applications. Several studies have examined the efficacy of these bioactive ingredients in cosmeceuticals, particularly in repairing damage to the hair scalp and shaft (Abelan *et al.*, 2022). The specific quantities and combinations of these ingredients determine the properties of these formulations, which are of interest for hair care purposes. These properties include antioxidant, anti-

inflammatory and antimicrobial activities, making essential oils promising ingredients for developing hair care products that promote hair health and address specific concerns (Abelan *et al.*, 2022; Youssef *et al.*, 2022).

Antioxidants play a crucial role in reducing oxidative stress in the body. In the context of hair growth, oxidative stress can damage hair follicles and cells, leading to hair loss or thinning. Antioxidants counteract this damage by scavenging free radicals and preventing them from harming the hair follicles (Beoy *et al.*, 2010; Serruya and Maor, 2021).

Hair follicles are highly sensitive structures that can be damaged by various factors, including environmental pollutants, UV radiation and inflammation. Antioxidants help protect hair follicles from damage caused by these external stressors. By neutralizing free radicals and reducing inflammation, antioxidants create a healthier environment for hair follicles to function optimally (Trüeb, 2021).

Antioxidants promote better blood circulation by protecting blood vessels from oxidative damage. Improved blood circulation ensures that hair follicles receive an adequate supply of nutrients and oxygen, which are essential for healthy hair growth. When blood flow to the scalp is optimized, it can enhance hair follicle function and promote hair growth (Abelan *et al.*, 2022; Saleem *et al.*, 2022).

Some antioxidants have been shown to inhibit the production of dihydrotestosterone (DHT), a hormone that can contribute to hair loss in individuals with androgenetic alopecia (male and female pattern baldness). By reducing DHT levels, antioxidants can help preserve hair follicles and promote hair growth in susceptible individuals (Baek *et al.*, 2017; Lourith and Kanlayavattanakul, 2013).

The present study considers castor oil (*Ricinus communis*), black seed oil (*Nigella sativa*), sunflower oil (*Helianthus annuus*), jojoba oil (*Simmondsia chinensis*), olive oil (*Olea europaea*) and pumpkin seed oil (*Cucurbita pepo*) as key ingredients of HG-HF oil. Castor oil acts as a natural emollient for the hair and helps in retaining moisture in the hair (Tessema, 2019). Black seed oil, also referred to as

kalojira oil, is a rich source of nigellone and thymoquinone which have positive effects on hair health by reducing hair loss, alleviating inflammation and providing relief from fungal allergies (Hasan *et al.*, 2012; Hussain and Musharraf Hussain, 2016). Sunflower oil possesses anti-inflammatory properties that have the potential to effectively eliminate dandruff and provide relief from scalp itchiness (Kumar *et al.*, 2012). Jojoba oil contains a rich combination of flavonoids, phenolic compounds, vitamins and minerals that nourish the hair. The strengthening properties of jojoba oil are believed to help prevent hair loss and promote thicker hair growth (Gad *et al.*, 2021). Topical application of oleuropein, a constituent of olive oil, induced hair growth (Almoselhy, 2021). Pumpkin seed oil contains a wealth of beneficial nutrients, including zinc, magnesium and omega-3 fatty acids which offer multiple advantages for the hair, such as moisturization, imparting shine, strengthening the hair strands and addressing hair damage (Ibrahim *et al.*, 2021).

The aim of the current study was to design and formulate a safe and effective hair growth oil formulation with adequate antioxidant activity from botanicals for promoting hair growth as well as treating alopecia and other hair fall related problems.

## Materials and Methods

*Chemicals and reagents:* Castor oil, sunflower oil, jojoba oil, olive oil, pumpkin oil and kalojira oil utilized in the formulation were obtained from local and international markets. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) reagent-grade potassium hydroxide (KOH) were obtained from Sigma Aldrich, USA.

*Evaluation of the prepared solutions:* Analysis of qualitative hair growth was performed by visual observation of initiation time for hair growth (time taken for initiating hair growth less hairy skin region) and completion time for hair growth i.e. minimum time taken to cover entirely with new hair. For physicochemical evaluation of the herbal formulation color, flavor, viscosity, refractive index and solubility

studies were conducted. Skin sensitivity or irritation test was employed to evaluate the irritation by the prepared formulations on the intact skin of volunteers. DPPH free radical scavenging activity test of the fixed oils represented by IC<sub>50</sub> value was performed for determining anti-oxidant activity. The IC<sub>50</sub> value, which is the concentration of a substance needed to scavenge or neutralize 50% of free radicals, indicates the strength of the antioxidant activity. The IC<sub>50</sub> values found for different oils were compared with the established values and then decided their activity status (Molyneux, 2004). Correlation of IC<sub>50</sub> value and antioxidant property has been presented in the table 1.

**Table 1. Correlation of IC<sub>50</sub> value and antioxidant property (Molyneux, 2004).**

IC <sub>50</sub> value (µg/ml)	Antioxidant property
<50	Very strong
50-100	Strong
100-150	Medium
150-200	Weak

### Antioxidant activity testing methods:

a. Preparation of DPPH solution: 2 mg of DPPH was weighed accurately and placed in a 100 ml beaker. Then methanol was added to make the solution 100 ml. Thus, the concentration of DPPH in the solution was 20 µg/ml. Then it was kept in a dark place.

b. Preparation of stock solution: 5 mg of each type of oil was measured and placed separately into six 50 ml beakers. Then the oil solutions were made up to 50 ml each using methanol. The final concentration of each stock solution was 100 µg/ml.

c. Preparation of samples: For each oil, the stock solution was diluted with methanol to prepare 10-100 µg/ml sample solutions. Then, 2 ml of each sample solution was taken into six different test tubes and 3 ml of a 20 µg/ml DPPH solution was added to each test tube. After that, the test tubes were kept in a dark place for 30 minutes. The absorbance was measured at 517 nm for each sample.

d. Preparation of positive control: 5 mg of ascorbic acid (AA) was measured and taken into a 50 ml beaker. Then, the stock AA solution was made up to 50 ml using methanol and the concentration was 100 µg/ml. Following that, the stock AA solution was diluted with methanol to prepare 10-100 µg/ml AA solutions. Then, 2 ml of the AA solution was taken into a test tube and 3 ml of a 20 µg/ml DPPH solution was added to the test tube. Then the test tube was kept in a dark place for 30 minutes and absorbance was measured at 517 nm.

e. Preparation of negative control: 2 ml of the methanol was taken into a test tube and 3 ml of a 20 µg/ml DPPH solution was added to it. Then the test tube was kept in a dark place for 30 minutes and absorbance was measured at 517 nm.

f. Calculation of IC<sub>50</sub> value: Inhibition of DPPH free radical was calculated for each oil using the following equation,

Inhibition of DPPH free radical, I (%) =

$$\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100\%$$

Where, A<sub>blank</sub> = Absorbance of blank and, A<sub>sample</sub> = Absorbance of sample

Then, the plot of % inhibition vs. concentration was constructed to determine the IC<sub>50</sub> (oil concentrations providing 50% inhibition of DPPH) value for each oil.

*Volunteer study:* 16 volunteers with hair loss, dandruff and other hair problems participated in the study. The volunteers were asked to use the oil once or twice daily according to their usage habits. They were directed to apply the oil on the scalp and massage with fingertips using a circular motion so that the oil reach the root of the hair and covers the entire scalp area. Additionally, they were advised not to wash the hair immediately or to comb vigorously after applying the oil. Moreover, they were not permitted to use any other oil or hair loss supplements during the course of the study. Data was collected regarding the use of this oil at the time points of 2 and 3 months. Ethical permission for the study was

obtained from the Ethical Review Board of the Faculty of Pharmacy, University of Dhaka. Informed consent was obtained from all participants.

## Results and Discussion

In respect of physicochemical properties, the prepared formulation was yellowish-black in color with pH (6.8) in accordance with human skin pH (6.8) which was neutral to slightly acidic (Table 2).

In this study olive oil, sunflower oil, kajojira oil, castor oil and jojoba oil showed IC<sub>50</sub> values of 3.79 µg/ml, 4.44 µg/ml, 8.34 µg/ml, 19.02 µg/ml & 19.90 µg/ml, respectively. As their IC<sub>50</sub> values are less than 50 µg/ml, they will perform as very strong antioxidant to scavenge free radicals and protect against oxidative damage (Table 3 and Figure 2). And as IC<sub>50</sub> value of pumpkin oil is 82.79 µg/ml which lies in the range of

**Table 2. Evaluation of physical parameters of hair growth-hair fall oil formulation.**

No	Parameter	Result
1	Color	Yellowish black
2	Odor	Characteristic
3	Appearance	Clear
4	Specific gravity	1.099
5	Viscosity	0.93
6	Acid value	4.5
7	pH	6.8
8	Sensitivity test	No irritation
9	Irritation test	No irritation
10	Grittiness	Smooth

**Table 3. IC<sub>50</sub> values of different fixed oils and ascorbic acid.**

Sample Name	IC <sub>50</sub> (µg/ml)
Castor oil (CAO)	19.02
Kajojira oil (KAO)	8.34
Sunflower oil (SUO)	4.44
Jojoba oil (JOO)	19.90
Olive oil (OLO)	3.79
Pumpkin oil (PUO)	82.79
Ascorbic acid (AA)	2.36

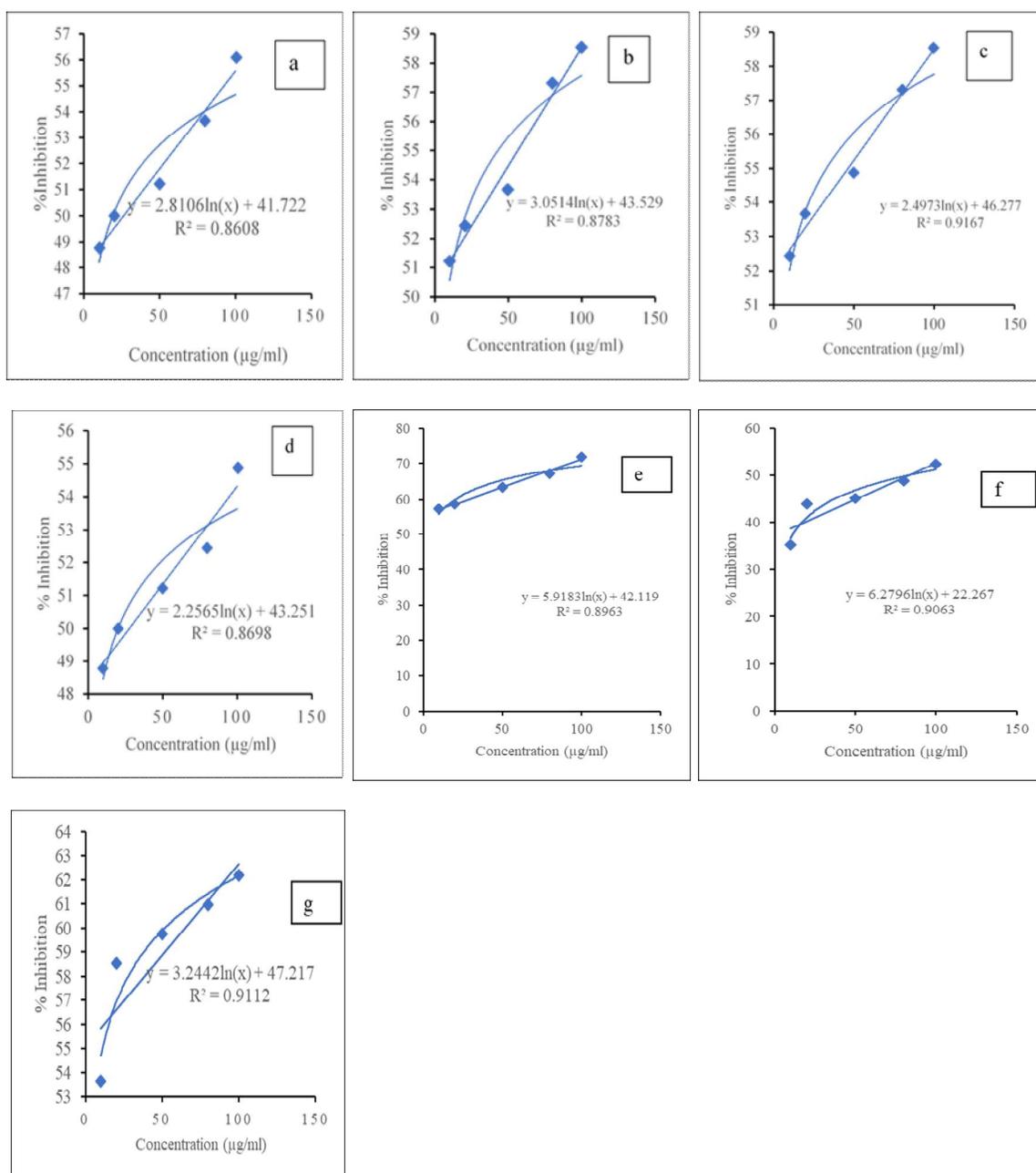


Figure 2. Free radical scavenging activity plot of % inhibition and predicted regression line of a. castor oil (*Ricinus communis*), b. kajojira oil (*Nigella sativa*), c. sunflower oil (*Helianthus annuus*), d. jojoba oil (*Simmondsia chinensis*) e. olive oil (*Olea europaea*), f. pumpkin oil (*Cucurbita pepo*) and g. ascorbic acid.

50 µg/ml -100 µg/ml, it will provide strong antioxidant effect. Thus the hair oil formulation may be said to have good antioxidant activity as DPPH free radical scavenging activity of every fixed oil showed good anti-oxidant effect (Table 3).

In the current study, we took 16 volunteers facing several hair problems including hair fall (81.25%), dry hair (43.75%), dandruff (31.25%), eczema (18.75%) etc. In the span of three months of observation, around 68.75% volunteers used the oil regularly and 25% used it sometimes (Figure 3a).

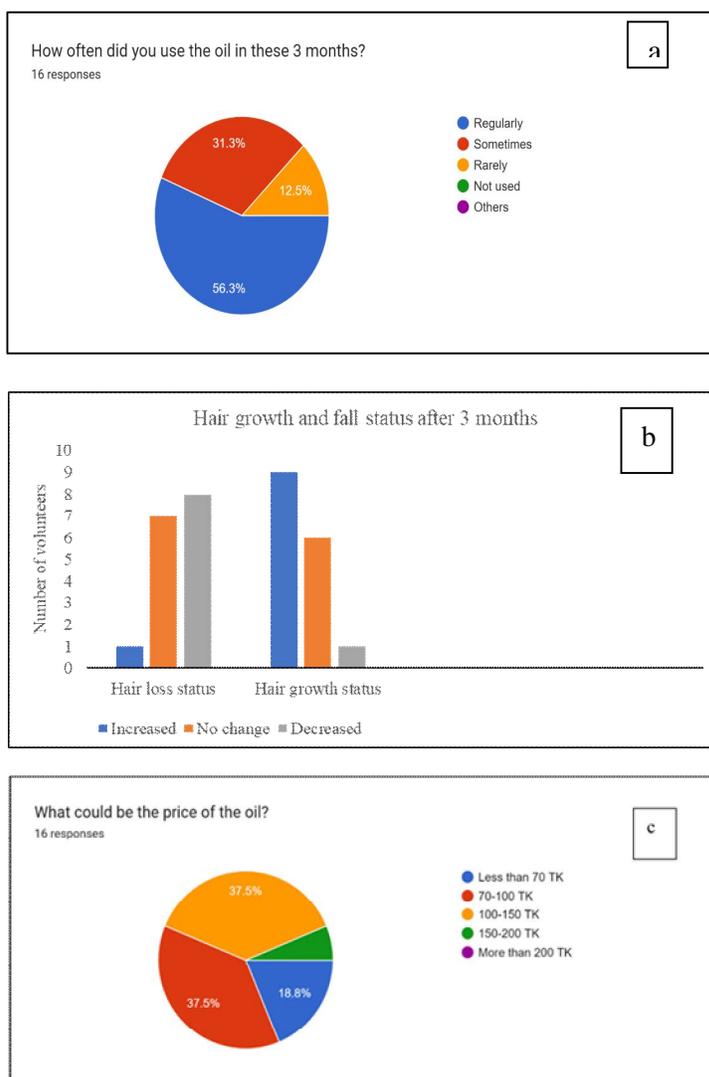


Figure 3. User status of the hair oil (after 3 months) a. user status, b. hair loss and hair growth change, c. perceived price.

*Effects on hair growth and hair fall:* It has been observed that 56.25% of the participants experienced increased hair growth (Figure 3b) and 50% volunteers reported decreased hair fall after using the oil for 3 months, with no remarkable side effects. Half of the participants reporting improvement in hair fall prevention and new hair growth rendered the hair oil formulation as promising if used properly.

*Additional benefits:* The additional benefits observed by 62.5% of the volunteers include getting shiny, strong and silky hair and anti-dandruff activity. Moreover, around 75% of respondents found the oil

aesthetic, user-friendly and got some benefits from using the oil.

*Side effects and other responses:* Two volunteers complained unpleasant odor and increased viscosity of the oil and only one volunteer felt mild irritation on the skin. However, 93.75% of participants claimed no side effect of using the oil. The participants were also asked to express their opinion on the probable commercial price of the product if marketed. Moreover, the feedback on the product's color, smell, texture and the perceived effects on hair was positive. The results are highlighted at table 4 and figure 4.

**Table 4. Evaluation of hair growth-hair fall oil formulation.**

Hair problems		User status of the oil		Observation (3 months)	
Problem	Response	Frequency	Response	Feedback	Response
Hair fall	13 (81.25%)	Regular	11(68.75%)	Aesthetic	7 (43.75%)
Dry hair	7 (43.75%)	Sometimes	4 (25.0%)	Hair fall decreased	3 (18.75%)
Dandruff	5 (31.25%)	Rare	1 (6.25%)	No effect	1 (6.25%)
Eczema	3 (18.75%)			Irritation	1 (6.25%)
No problem	1 (6.25%)			Complaining	2 (12.5%)
Additional benefits observed			Side effects observed		
Benefits	Response	Side effects	Response		
Shiny, smoother hair	4 (25.0%)	Itching	1 (6.25%)		
Strong hair observed	2 (12.5%)	No side effect	15 (93.75%)		
Silky hair obtained	3 (18.7%)				
No additional benefit	6 (37.5%)				
Effectiveness of the HG-HF oil					
Hair growth	Response	Hair fall status	Response	Anti-dandruff activity	Response
Increased	9 (56.25%)	Increased	1 (6.25%)	Effective	1 (6.25%)
Decreased	1 (6.25%)	Decreased	8 (50.0%)	Moderately effective	8 (50.0%)
No effect	6 (37.5%)	No effect	7 (43.75%)	Slightly effective	7 (43.75%)



Figure 4. Observation of change after usage of hair growth-hair fall oil formulation.

### Limitations of the study

Maintaining the product stability of the botanical oils can be challenging at times. Understanding all the effects of any cosmetic product can be time-consuming and user-dependent.

### Conclusion

Essential oils have gained significant interest and popularity among the increasing demand for herbal cosmetics in the market as they offer a range of potential benefits for the skin and hair. As hair fall is very common in Bangladesh, the project intended to find a fairly sustainable solution causing minimal side effects. The present study showed that the formulated HG-HF oil containing castor oil, black seed oil, sunflower oil, jojoba oil, olive oil, pumpkin seed oil as key ingredients provided numerous nourishing benefits to the hair and could be a safe alternative to improve hair growth and mitigate hair fall. As there were no adverse events associated with the study, it can be assumed that the test product is safe to apply topically.

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