

Formulation, Development and Evaluation of Herbal Preparation Containing Fenugreek Seeds and Avocado for Hair Nutrition

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ABSTRACT

Background: Loss of hair, dandruff, dryness, and premature greying are common complaints which are exacerbated by pollution, stress, malnutrition and the use of chemical-filled cosmetic products; synthetic hair care formulations, despite their effectiveness, are often linked with scalp irritation and drying with prolonged use, which has revived interest in herbal alternatives.

Purpose: The current research was conducted to design and test an herbal hair preparation containing fenugreek (*Trigonella foenum-graecum*) seed extract, a source of proteins, lecithin, saponins and nicotinic acid [1,14,15] with avocado (*Persea americana*) fruit oil, which is a source of oleic acid, essential fatty acids and vitamins A, D and E [8,9], with the objective of developing a synergistic and nutrient-dense product in the hair nourishment.

Procedures: Five trial formulations (F1-F5) were made by adjusting the ratio of ethanolic fenugreek seed extract (2-10% w/w) and cold-pressed avocado oil (5-25% w/w) into a coconut oil base, and were tested with regards to organoleptic properties, pH, viscosity, specific gravity, acid value, saponification value, spreadability, comparative hair-strand combability, potential skin irritation, and physical stability over the period of accelerated storage (40 ± 2 °C / 75 ± 5 RH, 90 days) [16].

Findings: The optimized batch (F4: 8% fenugreek extract, 20% avocado oil) had a homogenous look, had a scalp-acceptable pH (5.5 ± 0.09), appropriate viscosity (254 ± 7 cP) and spreadability, better hair-strand combability compared to untreated controls, matched the performance of a marketed reference product, and was not accompanied by irritation or phase separation over the 90-day study period.

Conclusion: The use of fenugreek-avocado herbal preparation combined is a stable, cosmetically acceptable and potentially useful, nutritive hair care ingredient to provide a rational support to the pharmacological and clinical trials of the formulation.

Keywords - Fenugreek, *Trigonella foenum-graecum*, Avocado, *Persea americana*, Herbal hair preparation, Hair nourishment, Accelerated stability.

How to cite this article: 1Ashok Kumar 2Prof.(Dr.)Anil Ahuja. Formulation, Development and Evaluation of Herbal Preparation Containing Fenugreek Seeds and Avocado for Hair Nutrition Int J Drug Deliv Technol. 2026;16(82s):1423-1432. DOI: 10.25258/ijddt.16.82s.163

.LIST OF ABBREVIATIONS

Abbreviation	Full Form
F1–F5	Formulation 1 to Formulation 5 (trial batches)
w/w	Weight by weight
v/v	Volume by volume
pH	Potential of Hydrogen
cP	Centipoise (unit of viscosity)
RH	Relative Humidity
SD	Standard Deviation
ANOVA	Analysis of Variance
rpm	Revolutions per minute
q.s.	Quantum sufficit (sufficient quantity)
°C	Degrees Celsius
g	Gram
mL	Millilitre
ICH	International Council for Harmonisation

1. INTRODUCTION**1.1 Overview of Hair and Scalp Physiology**

Human hair is a keratinized organ which develops through a hair follicle located in the dermis of the head and undergoes three physiological stages anagen (active growth), catagen (regression) and telogen (resting), and sheds and regenerates a new one. Its hair shaft is mostly made of the fibrous protein keratin, which is organized into an outer cuticle, a cortex and in thicker hairs a medulla. The cuticle is a protective sheath which defines shine, smoothness, mechanical and chemical resistance whereas the cortex gives tensile strength and elasticity. The health of the follicle and the micro-environment of the scalp, such as sebum production, blood supply and the nutritional status of the person can all be affected, either beneficially or detrimentally, by topically applied cosmetic preparations; all of which is closely associated with the health of the hair shaft.

1.2 Types of Hair Problems and their causes.

The most common hair and scalp complaints reported in all age groups include hair fall, thinning, dandruff, dryness, split ends and premature greying. These disorders can be caused by a variety of factors, which are usually interrelated, such as genetic predisposition (in the case of androgenetic alopecia), hormonal changes, nutritional deficiencies (especially of proteins, iron, zinc, and B-complex vitamins), psychological stress, exposure to ultraviolet radiation and environmental pollution, and repeated use of heat styling tools and severe chemical interventions, including bleaching, perming, or coloring. Since hair health is strongly correlated with self-image and social

confidence, even cosmetically insignificant factors like heightened hair loss or dryness could be a source of major psychological distress, which has maintained a large and expanding worldwide hair care product market based on preventing and treating these issues.

1.3 Limitations of Synthetic Hair Care Products

The majority of commercially-offered shampoos, conditioners and hair oils are based on synthetic surfactants (like sodium lauryl sulfate), silicones, mineral oil, synthetic fragrances, and preservatives in order to provide short-term sensory gratifications of foaming, shine and smoothness. Although these ingredients work in the short run, some of them have been linked to negative effects of long-/long-lasting/repeated use such as depletion of the natural lipid barrier of the scalp, contact irritation, clogging of the follicles and, occasionally, sensitization reactions in the case of some preservative systems. Moreover, products based on mineral oils or silicone-based products are more likely to cover the hair shaft, instead of penetrating it, providing only a superficial and temporary cosmetic enhancement without correcting nutritional or structural deficiencies of the hair and scalp.

1.4 A New Alternative: Herbal Cosmetics.

Due to increased consumerization of the possible adverse effects of synthetic compounds, the world has seen a pronounced switch towards herbal and plant-based cosmeceuticals. Plant-based oils, extracts, and pastes have been traditionally used to nourish hair in many traditional systems of medicine, including Ayurveda, Unani, and traditional Chinese medicine, and are generally seen as safer, less toxic and more biocompatible as well as more sustainable. The extra benefit of the herbal actives like fenugreek and avocado using a formulation science approach is that the herbs provide actually nutritive components, proteins, vitamins, essential fatty acids and antioxidants in place of merely coating the hair shaft, making them functional rather than cosmetic ingredients.

1.5 Fenugreek (*Trigonella foenum-graecum*): Botanical Profile and Traditional Use

Methi (Fenugreek *Trigonella foenum-graecum* Linn., family Fabaceae) is an annual herb that is commonly used as a culinary spice, as well as a traditional medicine plant, throughout South Asia, the Mediterranean, and North Africa [14,15]. The most pharmacologically important part of the plant is the seeds, which contain in particular the proteins the most (approximately 2530%), mucilaginous fiber,

steroidal saponins (especially diosgenin), flavonoids, alkaloids (including trigonelline) and nicotinic acid, as well as vitamins A, B1 and C [14,15]. Traditionally the paste of fenugreek seed or an aqueous/oil infusion of it is used on the head to condition the hair, treat dandruff and is traditionally thought to promote hair growth and slow greying. The interest in the pharmacological effects of fenugreek on hair care has been substantiated by reported in-vivo studies that report about enhanced hair length and follicular anagen-phase activity of topical application of fenugreek seed extract formulations [1,3] and favorable physicochemical stability of the formulations used [1,2].

1.6 Avocado (*persea americana*): Botanical Profile and Traditional Use.

Avocado (*Persea americana* Mill., family Lauraceae) is a tropical fruit tree, originally grown in Central America and today grown on a massive scale in both tropical and subtropical areas due to its richness in nutrients. The ripe fruit pulp is high in percent of oil, about half the dry weight of the fruit, which is mainly monounsaturated fatty acids, the oleic acid, with a trace of linoleic and palmitic acids, and is fortified with potassium, phytosterols and fat-soluble vitamins A, D and E [9]. Avocado oil is found in topical cosmetic formulations as an emollient and penetration enhancer, mainly due to a report that the oleic-acid-enriched composition of Avocado oil is more likely to penetrate the hair shaft and stratum corneum compared to most other vegetable oils, which reduces trans-cuticular water loss, flattens the cuticle surface, and protects against oxidative damage due to its inherent antioxidant (vitamin E and carotenoid) properties [8,9].

1.7 Reason as to why Fenugreek and Avocado should be combined.

Despite the fact that the use of the fenugreek seed extract and avocado oil as separate ingredients have been studied independently as hair care products, there is a relative lack of studies examining formulations that specifically aim to combine the protein-, saponin- and mucilage-rich profile of fenugreek with the fatty-acid- and vitamin-rich profile of avocado in hair care applications, which is why the topic of formulations integrating the two is underdeveloped in the field of pharmaceutical and cosmetic science literature. The hypothesis of the current work was that the two botanicals might work in a complementary fashion, with the fenugreek constituents offering nutritive and follicle-directed effects, and the avocado oil offering emollient, occlusive and antioxidant protection to the hair shaft, resulting in an overall

preparation with a more comprehensive nutritional profile than the individual ingredients.

1.8 Study Aim and Objectives.

The overall objective of the present study was to create and critically analyze an herbal hair preparation of fenugreek seed extract and avocado oil as a hair nutrient. The particular aims of the study were:

- The purpose of extraction, authentication and harvesting of fenugreek seeds and avocado fruit oil that can be used to develop a topical formulation.
- To conduct initial phytochemical screening on the fenugreek seed extract to be able to validate the existence of pharmacologically relevant constituents.
- To create and optimize an herbal hair oil product with different levels of fenugreek seed extract and avocado oil.
- To test the ready formulations regarding the organoleptic characteristics, physicochemical parameters, spreadability and rheological behavior.
- To determine the dermatological safety of the optimized formulation with a skin irritation (patch) study.
- To compare the conditioning effect of the optimized formulation on the tensile strength of the hair-strand against control conditions (untreated hair) and a commercial reference product.
- To determine the physical stability of the optimized formulation under accelerated storage conditions, in line with the traditional principles of stability testing.

2. LITERATURE REVIEW

Several past studies give scientific rationale behind the use of fenugreek and avocado, separately, in topical hair care products, and guide the design and evaluation plan used in the current study.

Swamy and co-workers developed a herbal hair gel based on ethanolic extract of fenugreek seed using Carbopol 934 as the gelling agent and tested the gel on physicochemical constants, spreadability, pH, viscosity and stability, as well as a hair growth experiment in mice, where topical application over 30 days resulted in an observable increase in hair growth on shaved skin without skin irritation [1].

The development and testing of a herbal hair gel to stimulate hair growth were reported by Regupathi who reported the standard evaluation parameters similar to those of a conventional topical gel, and the appropriateness of gel-based vehicles to deliver herbal actives to the scalp [2].

Wijaya and Mun'im tested the effectiveness of a fenugreek seed extract hair tonic in a rabbit model, and found that a 10% fenugreek extract hair tonic could induce statistically significant increase in hair growth activity relative to placebo with results comparable to a 2% minoxidil reference tonic with a Draize-based sensitivity assessment showing only potential of mild irritation [3].

Purewal and colleagues reported the formulation and testing of a multi-herb hair formulation that included fenugreek in the presence of other botanicals and reported that physical stability of the multi-ingredient hair formulation was satisfactory throughout the study period and showed the overall viability of multi-ingredient herbal hair formulations [4].

More recent evaluations of fenugreek as a cosmetic ingredient have also reported that, in spite of well-documented phytochemical profile and traditional reputation, the quantity of studies that have transformed fenugreek into fully-characterized topical cosmetic formulations is relatively small, and have recommended that that gap ought to be bridged by more standard formulation and evaluation methods, which the current study aims to contribute to [6].

In regards to avocado oil, a few reports detail its fatty-acid content, which is dominated by oleic acid with minor amounts of linoleic and palmitic acids, and its resulting use as emollient and conditioning agent in topical hair and skin preparations [9]. The reviews of avocado oil in hair cosmetics have cited its use in reducing friction and breakage of the cuticle, enhancing the ability to retain moisture in the hair fiber, and provide direct delivery of vitamin E and other antioxidants to the scalp and hair shaft, in addition to the research done on the fatty-acid derivatives of avocado oil and their protective action in other tissues with a keratinized and ciliated mucous membrane [8]. Zagade and colleagues used a mixture of natural oils and extracts to develop a herbal hair serum to be used as a general hair care product, and stated that combinations of botanical ingredients could be carefully chosen to be combined in one, cosmetically elegant vehicle without loss of physical stability, which supports the approach applied in the present study of combining fenugreek and avocado into one base [10].

This study involved the development and testing of an herbal hair serum by Tiwari et al and found that hair consistency and hair weight increased with application of this herbal hair serum and that only minor sensitivity was observed on first application

when appropriate preservative and antioxidant systems were employed [11].

Those reviews of avocado oil in cosmetic use have also highlighted that the quality and performance of avocado oil in cosmetic use requires the extraction technique to be a critical factor with cold-pressed avocado oil retaining a higher degree of heat-sensitive vitamins and antioxidants than solvent-extracted avocado oil or refined avocado oil, a factor that has had a direct influence on the adoption of cold-expression extraction used in the current research [9].

Combined, the available literature suggests the individual use of fenugreek seed extract and avocado oil as functional ingredients in hair care [1-4, 6, 8-11], but also shows that not many studies have combined and evaluated the two ingredients in a single formulation and subjected them to a structured, multi-parameter evaluation protocol including physicochemical characterization, dermal safety testing, comparative combability assessment, and accelerated stability testing side by side. This literature gap gave the particular impetus and scientific justification to the current study.

3. MATERIALS AND METHODS

3.1 Materials and Chemicals

Fenugreek seeds (*Trigonella foenum-graecum*), dried and fresh, ripe avocado fruits (*Persea americana*) were purchased in a local market. Coconut oil (base oil), ethanol (extraction solvent), glycerin, vitamin E acetate (antioxidant), methyl paraben and propyl paraben (preservatives) and perfume were obtained as analytical/cosmetic-grade reagents of a certified provider. All the reagents in the phytochemical screening and physicochemical analysis were of analytical quality.

Instrument/Equipment	Application in the study
Soxhlet apparatus	Extraction of fenugreek seed powder
Rotary evaporator	Concentration of the ethanolic extract
Digital pH meter	pH determination of formulations
Brookfield viscometer	Viscosity measurement
Specific gravity bottle (pycnometer)	Specific gravity determination
Mechanical stirrer with	Formulation of the

Instrument/Equipment	Application in the study
water bath	herbal hair oil
Analytical balance	Weighing of raw materials and reagents
Hot air oven	Drying of plant material and stability incubation control
Stability chamber	Accelerated stability studies (40 °C / 75% RH)

Table A. List of major instruments and equipment used in the study

3.2 Plant collection and Authentication.

Identification of the plant materials was carried out at the Department of Pharmacognosy of the mother institute and voucher specimens were deposited at the departmental herbarium as a reference in the future. Seeds and fruits that were not visibly contaminated with fungi or insects as well as those that had not deteriorated physically were extracted.

3.3 Preliminary Phytochemical Screening

Qualitative screening of the ethanolic extract of fenugreek seeds was performed by using conventional reagent-based tests reported in traditional pharmacognosy sources [14,15] to ascertain the presence of the major phytoconstituent classes that are likely to be active in terms of hair care activity:

- Alkaloids: Dragendorff's and Mayer's tests.
- Flavonoids: Shinoda (magnesium200mL–hydrochloric acid) test.
- Saponins: Froth formation test.
- Tannins and phenolics: Ferric chloride test.
- Proteins and amino acids: Biuret test and ninhydrin tests.
- Carbohydrates: Molisch's test and Fehling's test.

3.4 Preparation of Fenugreek Seed Extract

The seeds of fenugreek were washed, dried in shade and coarse powdered in a mechanical grinder. Continuous heat extraction of the powdered seeds (100 g) in a Soxhlet apparatus with ethanol (70% v/v) as the solvent was done in the Soxhlet system over a period of about 8-10 hours, or until exhaustive extraction of the solvent (as indicated by a siphon tube showing a colorless solution). The resulting extract was dried under reduced pressure in a rotary evaporator at regulated temperature (less than

50 C) to form a semisolid mass that was kept in an airtight amber-colored flask at 4 C until further use. The percent practical yield of the extract was determined against the weight of initial dried powder taken.

3.5 Preparation of Avocado Oil

Avocado fruits that were in good condition were washed, peeled and de-seeding was done, the homogenization of the pulp was done. The cold-expression (mechanical pressing) process to extract oil was selected as a method to minimize heating of fatty acids and vitamins, which are heat-sensitive, and filtration with muslin cloth and Whatman filter paper were used to eliminate particulate matter. Physicochemical constants of the extracted oil (acid value, saponification value, and refractive index) were compared to the values of physicochemical constants in the literature before the oil was incorporated into the formulation, to determine the quality of the oil against the literature values of cold-pressed avocado oil.

3.6 Formulation Development

The hot infusion method was used to prepare the herbal hair oil. The calculated amount of extract of fenugreek seed was triturated first with a little amount of coconut oil base so as to create a smooth dispersion which was then slowly added to the rest of the base oil with continuous stirring at 60–70 o C on water bath until it was well distributed. The inclusion of avocado oil was done at a lower temperature (less than 45 o C) to ensure that the unsaturated fatty acids and the vitamins in the oils were not degraded due to the elevated temperature. The supplemental antioxidant was vitamin E acetate and the preservatives (methyl and propyl paraben, 9:1) were used to prevent microbial contamination. The amount of perfume needed to create a cosmetically acceptable trace level was added, and the completed preparation filtered, cooled to room temperature and poured into amber glass bottles to prevent the degradation of the formulation caused by light. Table 1 summarizes five formulations (F1-F5) prepared by changing the concentration of fenugreek seed extract and avocado oil and keeping the other excipients the same.

Ingredient (% w/w)	F1	F2	F3	F4	F5
Fenugreek seed	2	4	6	8	10

Ingredient (% w/w)	F1	F2	F3	F4	F5
extract					
Avocado oil	5	10	15	20	25
Vitamin E acetate	0.5	0.5	0.5	0.5	0.5
Methyl paraben	0.18	0.18	0.18	0.18	0.18
Propyl paraben	0.02	0.02	0.02	0.02	0.02
Perfume	q.s.	q.s.	q.s.	q.s.	q.s.
Coconut oil base	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100	q.s. to 100

Table 1. Composition of trial herbal hair oil formulations (F1–F5)

3.7 Evaluation of the Formulated Preparation

The formulations obtained were assessed by the following parameters which were done in triple ($n = 3$) and expressed in mean and standard deviation (SD):

- Organoleptic assessment: The color, smell and physical appearance were evaluated visually and by the sense of smell and consistency with the skin was evaluated qualitatively.
- Homogeneity and grittiness: Evaluated by rubbing a small amount of the formulation between the thumb and index finger and visually/tactilely on a thin film on a glass slide.
- pH determination: Determined with a calibrated digital pH meter of a 10% v/v aqueous dilution of the formulation at room temperature.
- Viscosity: Measured in a Brookfield viscometer at 25 ± 1 of C and a speed of 50 rpm with a suitable spindle.
- Specific gravity: It is measured with a specific gravity bottle (pycnometer) against water at 25 ± 0.5 C.
- Acid value, saponification value: Measured using standard titrimetric procedures as found in pharmacopoeia [12,13].
- Spreadability: Determined by the time of separation between two glass slides under the given load, with a known amount of the formulation in between the plates; the spreadability was determined using the

standard formula of weight divided by length of the glass slide and time.

- Skin irritation (patch) test: Conducted on healthy human subjects with informed consent, by placing the optimized formulation on a small patch of the forearm and evaluating after 24 hours, either the presence or absence of erythema, edema, or irritation, using a Draize-type scoring [17] system.
- Hair-strand tensile strength / combability experiment: Hair strands (obtained with consent to a local salon) of human hair of uniform length were treated once per day over 15 days under the following conditions: untreated (control), optimized-formulation-treated, and marketed-product-treated; tensile strength was measured qualitatively by manual assessment of elongation/combability resistance, which was measured as the number of breakages per 10 strokes.
- Comparison with an existing marketed formulation: Optimized formulation was contrasted with one of the most popular commercial herbal hair oils regarding the pH, viscosity, and the index of combability, to put the performance in context to the existing commercial products.
- Accelerated stability test: The optimized formulation was kept in accelerated conditions (40 ± 2 C / 75-5% RH) 90 days as per the principle of the standard stability testing [16], and assessed 0, 30, 60 and 90 days in terms of organoleptic properties, pH, viscosity, and phase separation.

3.8 Ethical Considerations

Only after the written informed consent of healthy adult volunteers was received, the nature of the test formulation, procedure and their right to withdraw at any point with no consequences were made known to them, only at which point the skin irritation (patch) study was conducted. Participants that had a history of known skin allergies, dermatological or hypersensitivity to cosmetic ingredients were not allowed to participate. The hair strands used in the study on the combability were acquired with the consent of a nearby salon and did not involve any human subject procedure other than a normal hair collection process. The research was carried out in line with the principles of ethics in non-invasive cosmetic evaluation research on institutional level.

3.9 Statistical Analysis

Each evaluation was done in triplicate, and the results are presented in the form of mean SD.

Formulations were compared where appropriate by one-way analysis of variance (ANOVA) with $p < 0.05$ being statistically significant.

4. RESULTS AND DISCUSSION

4.1 Extraction Yield and Phytochemical Screening

A dark brown, semisolid, ethanolic extract of fenugreek seeds was yielded and it had typical odor with a practical yield range that is generally reported in Soxhlet ethanolic extraction of fenugreek seed (around 8-12% w/w) [1,2]. Phytochemical screening was performed to confirm the presence of saponins, flavonoids, alkaloids, proteins, and carbohydrates, which are in line with the reported phytoconstituents composition of *Trigonella foenum-graecum* seeds [14,15] and attest to their traditional application in hair care preparations. The cold-pressed avocado oil that was obtained after cold expression was pale yellow-green in color with an acid value and saponification value within the range of values reported in literature on the use of cold-pressed avocado oil as a cosmetic ingredient [9], which validates its applicability in the cosmetic industry.

Phytoconstituent	Test performed	Result
Alkaloids	Dragendorff's / Mayer's test	Positive
Flavonoids	Shinoda test	Positive
Saponins	Froth formation test	Positive
Tannins/Phenolics	Ferric chloride test	Positive (mild)
Proteins/Amino acids	Biuret / Ninhydrin test	Positive
Carbohydrates	Molisch's / Fehling's test	Positive

Table 2. Preliminary phytochemical screening of the ethanolic fenugreek seed extract

4.2 Organoleptic and Homogeneity Evaluation

All the five trial formulations were homogenous, not gritty and were light greenish-brown in color with the concentration of avocado oil and fenugreek extract increasing. It smelled as expected and could be used cosmetically after the addition of the perfuming agent, and consistency was between

a light, pourable oil (F12F2) and a slightly more viscous oil (F45). In all of the new fresh batches, no particulate matter or phase separation was noticeable.

4.3 Physicochemical Evaluation

Parameter	F1	F2	F3	F4 (Optimized)	F5
pH (10% aq. dilution)	5.8 ± 0.12	5.7 ± 0.10	5.6 ± 0.08	5.5 ± 0.09	5.4 ± 0.11
Viscosity (cP, 25 °C)	120 ± 4	168 ± 5	215 ± 6	254 ± 7	298 ± 8
Specific gravity	0.912 ± 0.004	0.915 ± 0.003	0.918 ± 0.004	0.921 ± 0.003	0.925 ± 0.005
Acid value	1.8 ± 0.15	2.0 ± 0.12	2.3 ± 0.18	2.5 ± 0.14	2.8 ± 0.16
Saponification value	188 ± 3	192 ± 4	196 ± 3	199 ± 4	203 ± 5
Spreadability (g-cm/sec)	18.2 ± 0.6	17.1 ± 0.5	15.8 ± 0.7	14.6 ± 0.5	13.2 ± 0.6

Table 3. Physicochemical evaluation of trial formulations (mean ± SD, n = 3)

All trial formulations were found to fall within the slightly acidic (5.4-5.8) physiological scalp and hair cuticle pH range and is likely to cause minimal irritation or disturbance to the cuticle on typical topical application. The viscosity and specific gravity rose with the concentration of avocado oil in the proportional measure to their concentration, which is in line with the fact that avocado oil is denser and contains more fatty-acids than the coconut oil base. The acid and saponification values were kept at reasonable levels of a topical oil-based formulation and were gradually rising with the avocado oil content, as it could be expected due to the presence of free fatty acid and glyceride components in it. The viscosity had a small negative effect on spreadability, although all batches, even the higher-concentration batches, exhibited spreadability values that could be easily used to apply to the scalp and hair topically.

Formulation F4 (8% fenugreek seed extract, 20% avocado oil) was chosen as the optimized batch based on a homogenous appearance, compatibility with other ingredients (physiologically compatible pH), acceptable viscosity and spreadability, as well as balanced concentration of both active ingredients.

4.4 Skin Irritation Study

The optimized formulation (F4) did not result in any visible erythema, edema, or other signs of irritation on the skin of the forearm of the volunteers during the 24-hour period, which means that this preparation is not irritant and is well-tolerated dermatologically in the conditions of the experiment. This observation is widely in line with the past reports on the topical preparations of fenugreek and avocado where the same have also reported the absence or mild irritation of patch testing [1,3,11].

4.5 Hair-Strand Comparative Evaluation and Hair-Strand Combability.

The optimized formulation applied daily during 15 days it resulted in the visually smoother hair-strand surface and fewer strand breakages per 10 combing strokes than untreated hair, which can be attributed to the conditioning effect of the combined action of

Parameter	Untreated hair	Optimized formulation (F4)	Marketed reference product
Breakages per 10 combing strokes	6 ± 1	2 ± 1	2 ± 1
Surface smoothness (qualitative)	Rough, frizzy	Smooth, glossy	Smooth, glossy
pH	—	5.5 ± 0.09	5.6 ± 0.10
Viscosity (cP)	—	254 ± 7	310 ± 9

Table 4. Comparative hair-strand combability and physicochemical evaluation

4.6 Accelerated Stability Study

The developed optimized formulation maintained its organoleptic properties, pH, and viscosity within close ranges during the 90-day storage period (accelerated) with no evidence of visible phase separation, precipitation or rancidity indicating acceptable physical stability. The small decrease in viscosity and pH with time is probably due to slow oxidative reactions in the unsaturated fatty acid fraction added by avocado oil, and may be reduced further by the addition of other antioxidants or opaque, oxygen impervious packaging in a commercial formulation.

4.7 Effect of Fenugreek Seed Extract Concentration

A gradual deepening of the color of the formulation and a slight rise of the viscosity were observed with the increase in concentration of the fenugreek seed extract to 10% (F5) due to the larger share of mucilaginous and proteinaceous solids added by the extract. Recipes with increased extract levels (F4-F5) were also associated with a slightly stronger characteristic odor, and required a slightly higher ratio of perfusing agent to be cosmetically acceptable. Even at the highest extract concentration that was tested, there was no adverse impact on homogeneity or filterability of the formulation, showing good compatibility of the extract with the coconut oil base in the conditions of processing.

4.8 Effect of Avocado Oil Concentration

The concentration of avocado oil was also discovered to be the most important factor influencing viscosity, specific gravity, acid value and saponification value of the trial formulations with all the four parameters rising in a concentration-dependent manner starting with F1 (5% avocado oil) to F5 (25% avocado

fenugreek proteins/saponins and avocado-oil fatty acids. The optimized formulation was found to have a more or less similar combability index and pH when compared to a leading marketed herbal hair oil, with slightly lower viscosity indicating less greasy after-feel.

Table 5. Accelerated stability data for the optimized formulation (F4), 40 ± 2 °C / 75 ± 5% RH

Storage period	Appearance	pH	Viscosity (cP)	Phase separation
Day 0	Homogeneous	5.5 ± 0.09	254 ± 7	None
Day 30	Homogeneous	5.5 ± 0.10	251 ± 6	None
Day 60	Homogeneous	5.4 ± 0.11	248 ± 8	None
Day 90	Homogeneous	5.4 ± 0.10	244 ± 7	None

oil). This is also in line with the inherent physicochemical characteristics of avocado oil which is heavier and fatty acids and glycerides when compared with the coconut oil base. Sensory analysis revealed that formulations with concentrations of avocado oil higher than about 20 percent (F4 F5) gave a more rich, emollient after-feel to the skin when applied, whereas formulations with concentrations lower than about 15 percent (F1 F3) resulted in a lighter, faster-absorbing film; this compromise between richness and lightness supported the choice of a middle-high concentration (20, as used in F4) in the optimized formulation.

4.9 Overall Discussion

The combination of avocado oil and fenugreek seed extract in one herbal hair product seems to provide an extra benefit of the protein-, saponin- and lecithin-rich profile of fenugreek with follicular nourishment and conditioning of the hair shaft, and the oleic-acid- and vitamin-rich avocado oil with emollient, moisture-binding and antioxidant effects that prevent hair cuticle breakage and environmental damage. The physicochemical analysis showed that a stable, homogenous, non-irritated, and cosmetically acceptable formulation was possible in the concentration ranges investigated and the comparative combability data indicated that the optimized formulation was comparable to an existing marketed product and was also associated with a relatively light texture. Combined, these findings indicate the technical viability of integrating these two botanicals to form one hair care preparation. However, the current analysis was limited to physicochemical characterization, a short term patch-level irritancy study, an in-vitro combability test and 90 days accelerated stability testing; controlled clinical trials of long-term hair growth, hair

fall reduction, dandruff control, and consumer acceptability during prolonged use would be required before any definitive therapeutic or cosmetic claim could be made.

5. STUDY Limitations.

Although this study offers a systematic physicochemical and preliminary safety analysis of the fenugreekavocado herbal hair preparation, it is possible to note some limitations. The skin irritation test was performed on few healthy volunteers in a short time period and is not a replacement of a complete dermatological safety test on a variety of skin types. The hair-strand combability experiment has been conducted in vitro on free hair strands and, despite suggesting the existence of a conditioning effect, is not able to recapitulate the complexity of the physiological environment of the living scalp and follicle. Likewise, the active constituents of the fenugreek extract as well as the avocado oil were not quantitatively standardized (e.g. by high-performance liquid chromatography or gas chromatography), which could hamper batch-to-batch reproducibility in a scaled up or commercial context. Lastly, stability data is only available to 90 days under accelerated conditions; actual stability research to the desired shelf life of the product would be necessary prior to commercial launching.

6. CONCLUSION

A herbal hair conditioner made with fenugreek seed extract and Avocado oil was developed and tested successfully. The formulation with 8% fenugreek seed extract and 20% avocado oil (F4) had the best balance of physicochemical characteristics, such as suitable viscosity and spreadability, scalp-compatible pH, absence of skin irritation, greater combability of hair strands compared to untreated hair, similar performance to a commercial reference product, and acceptable physical stability in accelerated storage conditions. These results suggest that a stable, non-irritant, nutritionally-enriched herbal formulation to nourish hair using fenugreek and avocado as the active components could be developed and that the combination could have a complementary follicular and cuticular effect not attained by either ingredient alone.

7. FUTURE SCOPE

The current results can be further generalized by future work to quantitatively estimate the key active components (including diosgenin and total saponin content in the fenugreek extract, and fatty-acid profiling of the avocado oil using gas chromatography) to allow batch-to-batch standardization. Clinical trials that evaluate the hair growth parameters and hair

fall reduction and dandruff control with longer treatment periods and the formulation into alternative dosage forms such as gels, serums or shampoos and long tests (real-time) would further help in strengthening the evidence base as well as making possible commercial and clinical translation of the formulation.

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