

FORMULATION AND EVALUATION OF ANTI AGING AND ANTI WRINKLE FENUGREEK EXTRACT CREAM

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ABSTRACT:

Aging of the skin is a complicated biological process that is impacted by both external and internal factors. Many anti-aging techniques have been created in recent years because to the belief that one of the primary variables indicating an individual's general "well-being" and perception of "health" is their skin's health and beauty. This article aims to discuss the most significant anti-aging techniques dermatologists currently employ, such as preventive measures, cosmetic techniques, topical and invasive techniques, as well as systemic medicinal medications.

KEYWORDS: Melanin, erythema, skin, skin moisture, sebum.

INTRODUCTION

Skin aging is a part of the natural "aging mosaic" that occurs in humans and takes varied forms throughout time in various organs, tissues, and cells. The skin offers the first visible indications of the passage of time, whereas the aging signals of internal organs are concealed by the ambient "eyes."

Exogenous or extrinsic variables (chronic light exposure, pollution, ionizing radiation, chemicals, toxins) and endogenous or intrinsic factors (genetics, cellular metabolism, hormone and metabolic processes) combine to influence the complicated biological process of skin aging. Collectively, these elements result in cumulative physiological and anatomical changes, as well as gradual modifications to every layer of the skin.

Anti-aging, antioxidants, hormone replacement treatment, laser, peeling, fillers and botulinum toxin in the look of the skin, particularly on sun-exposed areas.^[2-12] A thicker epidermis, mottled coloring, deep wrinkles, laxity, dullness, and roughness are typical characteristics of prematurely photo-aged skin, as opposed to thin and atrophic, finely wrinkled, and dry intrinsically aged skin.^[13-18] Sagging is a result of the skin's elasticity gradually declining.^[19] In elderly people, slower wound healing and less successful desquamation are correlated with slower epidermal turnover rate and longer cell cycle. When scheduling aesthetic procedures, this information is crucial.^[20]

However, a lot of these characteristics are intended to be used with products or processes that quicken the cell cycle, with the idea that a faster turnover rate will yield improvement in skin appearance and healing. The look of the skin will improve and wound healing will happen more quickly with a higher turnover rate.^[21] Wrinkles may result from a substantial loss of fibrillin-positive structures^[22] and a decrease in collagen type VII (Col-7) content, which weakens the connection between the dermis and epidermis of extrinsically aged skin.^[23] Solar elastosis is a characteristic of aging skin exposed to the sun. In spite of the same collagen production, greater collagen degradation by different matrix metallo proteinases, serine, and other proteases may be the cause of the sparse distribution and decreased collagen content in photoaged skin.^[24-28]

It has been demonstrated that an increase in the ratio of Col-3 to Col-1 is largely caused by a major loss of Col-1, which is why older skin has uneven and disorganized collagen.^[29] The total amount of collagen in each skin region.

It is possible that the increased collagen deg surface, which is known to diminish by around 1% annually, is the cause of the sparse distribution and decrease in collagen content in photoaged skin.^[30] One of the main components of the dermal skin matrix that aid in water binding is glycosaminoglycans (GAGs). GAGs may be linked to aberrant elastotic material in photoaged skin, which would prevent them from performing as intended.^[31] In skin that ages naturally, the overall amount of hyaluronic acid (HA) in the dermis stays constant, whereas the amount of HA in the epidermis decreases significantly.^[32]

Collagen, elastin, and glycogen-boosting agents (GAGs) are the three main structural elements of the dermis. These components have been the focus of most anti-aging research and efforts related to skin care, ranging from "wrinkle creams" to other filling agents.^[33]

The appearance of facial aging on its whole is linked to the main structural components of the skin, like elastin and collagen, to stop wrinkles from forming. Some products do encourage the natural production of these chemicals, with the exception of elastin-enhancing ones, even if the technology needed to appropriately carry them into the skin has not yet been created. Reducing inflammation with topical or systemic antioxidants is another essential strategy to avoid wrinkle formation; these antioxidants should be used in conjunction with sunscreens and retinoids to maximize their preventive benefits.

ANTIOXIDANTS IN THE SYSTEM AND PHOTOPROTECTION

Extrinsic skin aging, or photoaging, is a symptom of chronic photodamage of the skin. Reactive oxygen species (ROS) produced by UV light and DNA photodamage are the first molecular processes that cause the majority of the common histological and clinical signs of chronic skin photodamage. Bending and pigmentary alterations are thought to be the most significant cutaneous symptoms of premature photoaging. Avoiding the sun, using sunscreens to protect against UV rays, retinoids to inhibit the synthesis of collagenase and increase the production of collagen, and using antioxidants, especially in combination, to lower and neutralize free radicals (FR) are some of the strategies used to prevent photoaging.

Pharmacological Topical Agents with Anti-Aging Characteristics

Antioxidants and cell regulators are the two main categories of substances that can be included in anti-aging creams. By lowering the amount of FR in the tissues, antioxidants such as vitamins, polyphenols, and flavonoids slow down the deterioration of collagen. Growth factors (GF), retinols, and peptides are examples of cell regulators that directly affect and alter the synthesis of collagen and collagen metabolism.

The most significant antioxidants are vitamins C, B3, and E because of their small molecular weight, which allows them to permeate the skin. It has been demonstrated that water-soluble, heat-labile local L-ascorbic acid (vitamin C) at concentrations of 5–15% has an anti-aging effect on the skin by promoting the production of Col-1 and Col-3, as well as collagen-producing enzymes and matrix metalloproteinase (MMP) 1 (collagenase 1) inhibitors. Clinical research has demonstrated that the combination of vitamins C and E provides greater antioxidant protection than either vitamin taken alone.^[33] Vitamin B3, niacinamide, controls cell metabolism and regeneration and is utilized as an anti-aging treatment at a concentration of 5%. According to certain research, after three months of topical therapy, elasticity, erythema, and pigmentations have been noted. When added to skin products, vitamin E (α -tocopherol) exhibits anti-inflammatory and antiproliferative properties at doses ranging from 2 to 20%. It works by making the skin smoother and enhancing the stratum corneum's capacity to retain humidity, speed up the process of epithelialization, and support skin photoprotection. The benefits are not as great as those of vitamins B3 and C.³⁸ In the near future,

Invasive techniques

Numerous in-office techniques exist, the majority of which aim to "resurface" the epidermis by removing damaged tissue and replacing it with reformed skin layers. Occasionally, these procedures also stimulate the production of new collagen. It's probable that telomerase, cytokines, and growth factors will all eventually have their effects harnessed through innovation and technological improvement in the rapidly developing disciplines of gene therapy and tissue engineering.

Chemical Peels

Chemical peels are procedures used to chemically ablate certain skin layers, resulting in tighter, more even skin as a consequence of the skin's repairing and regenerating systems following inflammation of the dermis and epidermis. Three types exist for chemical peels.^[33] Deep peels (TCA > 50%, phenol) enter the lower reticular dermis, while medium-depth peels (TCA above 30 to 50%) reach the upper reticular dermis and superficial peels (α - β -, lipo-hydroxy acids (HA), trichloroacetic acid (TCA) 10–30%) exfoliate epidermal layers without reaching beyond the basal layer. Peeling depth is influenced by the material's concentration and pH in addition to the substance itself, of the remedy and the application's timing.



Figure 1: 45-y-old female with signs of photoaged skin: dyschromia of the skin, multiple lentigenes before skin antiaging skin treatment .



Figure 2: 45-y-old female with signs reduced signs of photoaged skin after one treatment with IPL with 550 nm cut-off filter.

IPL, lasers, and radiofrequency (RF) devices for skin resurfacing, tightening, and rejuvenation

- IPL: Uses a bright light to target elastin and improve skin tone by reducing redness and sunspots. It can also help with hyperpigmentation, rosacea, and inflammation.
- RF: Uses controlled heat to stimulate collagen production and tighten skin. RF devices can be monopolar, bipolar, or fractional:
 - Monopolar: Uses a single electrode to heat the skin
 - Bipolar: Uses two electrodes on a handpiece to deliver current to the skin
 - Fractional: Uses microneedles to create controlled injuries and stimulate collagen production
- Lasers: Can be used to slow or reverse signs of aging.

"Subsurfacing," also known as nonablative skin rejuvenation, is a low-risk, quick recovery technique that can repair structural aging changes in the skin without compromising cutaneous integrity.⁷⁷The proposed mechanism of action involves the selective denaturalization of cutaneous collagen caused by heat, which in turn triggers reactive synthesis. Since rejuvenation is a controlled type of skin injuring intended to achieve a more youthful appearance once the wound heals, the term "nonablative skin rejuvenation" is imprecise.

Treatment options for photoaged skin include improving the dermal and subcutaneous layers as well as treating ectatic vessels, erythema, uneven pigmentation, and pilosebaceous alterations (Type I) ageing (Type II).Two main processes can be used to selectively damage the epidermis and superficial dermis: (a) using mid-infrared (IR) lasers to target discrete chromophores

in the dermis or at the dermal-epidermal junction, or (b) using other methods



Figure 3: (A) before laser treatment (B) After laser treatment

Histological slices of skin taken both before and after treatment with the various IPL devices have demonstrated the production of new collagen in the reticular and papillary dermis in addition to an increase in fibroblasts and a corresponding decrease in solar elastosis. In the event that vascular and/or pigment abnormalities improve right away, the collagen remodeling reaction

Skin Rejuvenation Injectables and Dermal Fillers

Increased fibroblast biosynthetic capacity leads to the restoration of an ideal physiological environment, increased cell activity, improved hydration, and the synthesis of collagen, elastin, and HA (hyaluronic acid). This is the aim of skin biorejuvenation. Microinjections of single-active-ingredient products or combinations of highly absorbable and biocompatible substances, such as GF, amino acids, autologous cultured fibroblasts, vitamins, minerals, nutrients, and homeopathic remedies, could produce the intended results in the superficial dermis.¹¹⁶In vitro, the different formulations can cause fibroblasts to undergo remarkably different molecular and cellular processes.

To clarify whether and how the cellular and molecular processes are involved in facial skin renewal in people, further thorough research is necessary.

Whether these procedures are as effective in vivo, regardless of the patients' ages. Additionally absent is the proof of concept, which includes long-term efficiency and ideal injection techniques.

Fillers are substances that are injected under or within the skin to enhance its appearance through soft tissue augmentation. In addition to synthetic or pseudo-synthetic implants (silicone, polymethacrylate microspheres, poly-L-lactic acid, calcium hydroxylapatite microspheres suspended in aqueous polysaccharide gel, alkyl-imide gel polymer), there are autologous (fat, cultured human fibroblasts), collagen (bovine-derived, human-derived from tissue culture), and HA (nonanimal stabilized or viscoelastic HA from bacterial fermentation). These can be divided into three categories: semipermanent (lasting 1-2 years), permanent (lasting more than 2 years), and temporary materials

Platelet-rich plasma (PRP) that is autologous

The use of autologous platelet-rich plasma (PRP) for skin rejuvenation has gained popularity. Fresh whole blood, which has a high concentration of platelets, is used to make PRP.¹⁶⁰ The α -granules of concentrated platelets activated by aggregation inducers secrete several growth factors (GF), such as insulin-like growth factor (IGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF).¹⁶¹ By binding to particular cell surface receptors, these factors are known to influence activities such as cell migration, adhesion, proliferation, and differentiation. They also encourage the buildup of extracellular matrix (ECM).^[43] Research indicates that platelet-rich plasma (PRP) can stimulate the activation of fibroblasts, which in turn can induce the manufacture of collagen and other matrix components, thereby renewing the skin. The molecular pathways behind PRP-induced wound healing processes, however, remain still unknown and experimental studies confirming the effects of PRP on aged fibroblasts are very limited.

Botulinum toxin (BTX)

Botulinum toxin cannot stop the aging process of the skin and has no effect on the texture of the skin. However, by managing some dynamic facial lines and wrinkles, regular BTX injections can assist to slow down the obvious aging process.



Figure 4: Patient showing glabellar and crow's feet wrinkles. (A) pre-injection, (B) after injection with botulinum toxin.

The underlying cause of crow feet, glabellar lines, and accentuated frown lines—excessive nerve stimulation—is not addressed by the current therapeutic options, which include surgery and implants. Because of its mode of action, BTX is a perfect agent to focus on the primary cause of these dynamic lines:

MATERIAL AND METHOD

List of Active and Inactive Material ^[129]

Table: 1 list of material used

Sr. No	Name	Type
	Fenugreek seed Powder	Active
	Span 60	Inactive
	Liquid paraffin	Inactive
	Ethanol	Inactive
	Sodium Dihydrogen Phosphate Dihydrate	Inactive
	Tween 80	Inactive
	Carbopol940	Inactive
	Methyl Paraben	Inactive
	Propylene glycol	Inactive
	Citric Acid	Inactive
	Sodium Hydroxide	Inactive

Formulation:

Different quantities of various inactive materials are used with definite quantity of fenugreek for stable cream preparation. The quantity used has been given in the following **table: 8** for twelve samples FG1, FG2, FG3....FG12.

Procedures:

Cream was prepared by physical dispersion method using differentration of Carbopol 940 and Span 60. The Cream phase in the formulations was prepared by dispersing Carbopol 940 in purified water with constant stirring at a moderate speed using mechanical shaker, then the pH was adjusted to 6–6.5 using sodium hydroxide. The oil phase of the cream was prepared by dissolving span 60 in light liquid paraffin while the aqueous phase was prepared by dissolving tween 80 in purified water.

Fenugreek extract was formed by dissolving and boiling powder in water for 30 minutes filter it. To filtrate add Methyl paraben which was dissolved in propylene glycol both solutions were mixed with the aqueous phase. Both the oily and aqueous phases were separately heated to 70–80 °C, then the oily phase was added to the aqueous phase with continuous stirring until it got cooled to room temperature. The obtained Cream is then undergo stirring at 1500 rpm for and 30mins. Then add other inactive ingredients and maintain pH with citric acid and make up the volume 100 ml with water and stirrer for 30 minutes for making smooth Cream

Evaluation Parameters

Physical evaluation

The formulated fenugreek cream should be smooth, homogenous and free from grittiness. For this purpose, color, phase separation and homogeneity is observed of various samples.

Spreadability

Spreadability is checked by using Arvouet-Grand Method.

Spreadability of creams was determined by pressing 1 g of sample between two 20X20cm horizontal plates, the upper of which weighed 125 g. The spread diameter was $\phi \leq 50$ mm and semifluid cream should show spreadability diameter $\phi > 50$ mm but < 70 mm. Measured the spreadability of formulations was done in the triplicate and the average value was calculated.

Viscosity

The viscosity of the formulated batches was determined using a cone and plate viscometer with spindle 63 (Brookfield Engineering Laboratories). The assembly was connected to a thermostatically controlled circulating water bath maintained at 25 °C. The formulation whose viscosity was to be determined was added to a beaker covered with thermostatic jacket. Spindle was allowed to move freely into the Cream. The spindle no.63 was dipped in cream sample rotated at 10rpm and $20 \pm 1^\circ\text{C}$ temperatures for 15 min. The reading in triplicate was noted. Viscosity in centipoise (cp) was measured.

pH

1 % aq. a solution of cream formulation was prepared and stored for 2 hours, pH was determined using a digital pH meter. The pH of each cream formulation was done in the triplicate, average value and \pm standard deviation was calculated. The pH of the cream formulations should lie in the range of 5 ± 0.2 to 7.5 ± 0.2 which lies in the normal range of skin. For stable and non irritating formulation, pH should lie in the limit.

Homogeneity and Grittiness

All developed Cream formulations were allowed to set in a suitable container and tested for homogeneity A small quantity of Cream was pressed between the thumb and the index finger. The consistency of the cream was noticed (whether homogenous or not), if there was any coarse particles appeared on fingers. Also, the homogeneity could be detected when a small quantity of the cream was rubbed on the back of the hand. The grittiness of the prepared cream was also observed in the same manner.

Assay Percentage (Drug content)

Method of analysis

Standard Solution-

100mg of Fenugreek powder dissolved in 50 ml water boil it then make up volume 100ml with methanol. From the above solution 10 ml was pipette into a 100ml volumetric flask and made upto 100ml using methanol to get a stock solution of 100 µg/ml. From this stock solution, a 10 ml was pipette and transfer into a 100ml volumetric flask and made up to mark with methanol to get a concentration in the range of 10 µg/ml.

Test Solution-

1ml of Cream eq to 500mg of Fenugreek extract is dissolved in 100ml of methanol. From the above solution 2 ml was pipette out into a 100ml volumetric flask and made upto 100ml using methanol to get a stock solution of 100 µg/ml. From this stock solution, a aliquots of 10 ml were pipette out into a 100ml volumetric flask and made up to mark with methanol to get a concentration in the range of 10 µg/ml. The absorbance of the resulting solution was then measured at 295.5nm using UV Double beam spectrophotometer against methanol as blank using UV-spectrophotometer.

In vitro studies

In-vitro drug release studies revealed that the release of Fenugreek from different formulations varies with characteristics and composition of excipients. The comparison of the drug release profile of all formulations showed that formulation is observed with maximum drug release of 99.11%. is is based upon quick release of drug from the formulation into the skin. In vitro studies are needed to be performed for the drug release and skin permeability.

Stability studies

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of environmental factors such as temperature, humidity and light. The stability of developed Cream was established over a period of 90 days' time intervals, the optimized formulation was kept at $40^{\circ}\text{C} \pm 2/\text{RH}70\% \pm 5$ and was evaluated for organoleptic properties, pH and drug content and the results are mentioned in the Table 8. Results indicate that the formulation was stable and no significant changes were observed. After short listing the samples which are not upto the mark, moving forward for stability testing.

RESULT AND DISCUSSION

FORMULATION

Formulation of Cream loaded with Fenugreek as Fenugreek powder

Table 2: Optimization of Fenugreek cream**Optimization of Cream**

Ingredients	Quantity											
	FG1	FG2	FG3	FG4	FG5	FG6	FG7	FG8	FG9	FG10	FG11	FG12
Fenugreek powder	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%
Span 60	0.5%	1%	1.5%	2%	0.5%	1.7%	1.5%	2%	0.5%	1%	1.5%	2%
Carbopol940	0.01%	0.02%	0.03%	0.04%	0.01%	0.02%	0.03%	0.04%	0.01%	0.02%	0.03%	0.04%
Liquid paraffin	0.1%	0.1%	0.1%	0.1%	0.8%	0.3%	0.3%	0.3%	0.5%	0.4%	0.5%	0.5%
Ethanol	0.02%	0.01%	0.02%	0.04%	0.02%	0.02%	0.01%	0.02%	0.02%	0.05%	0.02%	0.02%
Sodium Dihydrogen Phosphate Dihydrate	0.1%	0.12%	0.1%	0.13%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Tween 80	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%
Methyl Paraben	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%
Propylene glycol	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Citric Acid	0.02%	0.01%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%	0.02%
Sodium Hydroxide	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%	0.03%
Distilled Water	q.s. 100g	q.s. 100g	q.s. 100g	q.s. 100g	q.s. 100g	q.s. 100g	q.s. 100g	q.s. 100g	q.s. 100g	q.s. 100g	q.s. 100g	q.s. 100g

Process of Formulation

Cream manufacturing need mechanical equipment. Micro-fluidizers, homogenizers, and ultrasonicators can provide these forces, but they are expensive and produce high working temperatures, which are in an appropriate for drugs that are thermolabile. Cream was prepared

by physical dispersion method using differentiation of Carbopol 940 and Span 60. The Cream phase in the formulations was prepared by dispersing Carbopol 940 in purified water with constant stirring at a moderate speed using mechanical shaker, then the pH was adjusted to 6–6.5 using sodium hydroxide. The oil phase of the cream was prepared by dissolving span 60 in light liquid paraffin while the aqueous phase was prepared by dissolving tween 80 in purified water. Fenugreek extract was formed by dissolving and boiling powder in water for 30 minutes filter it. To filtrate add Methyl parabene which was dissolved in propylene glycol both solutions were mixed with the aqueous phase. Both the oily and aqueous phases were separately heated to 70–80 °C, then the oily phase was added to the aqueous phase with continuous stirring until it got cooled to room temperature. The obtained Cream is then undergo stirring at 1500 rpm for and 30mins. Then add other inactive ingredients and maintain pH with citric acid and make up the volume 100 ml with water and stirrer for 30 minutes for making smooth Cream.

Evaluation of optimized Cream

Physical Evaluation

Table 3: Physical evaluation of Fenugreek cream formulation

Batch No.	Color	Phase separation	Homogeneity
FG1	Off White	No	Good
FG2	Off White	No	Not Good
FG3	Off White	No	Good
FG4	Off White	No	Good
FG5	Off White	No	Not Good
FG6	Off White	No	Good
FG7	Off White	No	Good
FG8	Off White	No	Not Good
FG9	Off White	No	Good
FG10	Off White	No	Not Good
FG11	Off White	No	Good
FG12	Off White	No	Good

Discussion: All the prepared fenugreek cream formulations were off white preparations with a smooth and homogeneous appearance. Results have been discussed in **Table 3**.

Spreadability

Method : Arvouet-Grand Method

Spreadability of creams was determined by pressing 1 g of sample between two 20X20cm horizontal plates, the upper of which weighed 125 g. The spread diameter was $\phi \leq 50$ mm and semifluid cream should show spreadability diameter $\phi > 50$ mm but < 70 mm. Measured the spreadability of formulations was done in the triplicate and the average value was calculated.¹⁷ The spreadability of the prepared Cream was found to be 26.15 g.cm/sec. This indicates that the Cream can get easily spread out when applied on skin.

Spreadability achieved by Arvouet-Grand Method

Table 4: Spreadability of Fenugreek Cream

Batch No.	(g.cm/s)
FG-1	28.17
FG-2	24.32
FG-3	21.15
FG-4	18.81
FG-5	24.32
FG-6	24.325
FG-7	24.81
FG-8	18.91
FG-9	24.78
FG-10	10.44
FG-11	22.74
FG-12	19.79

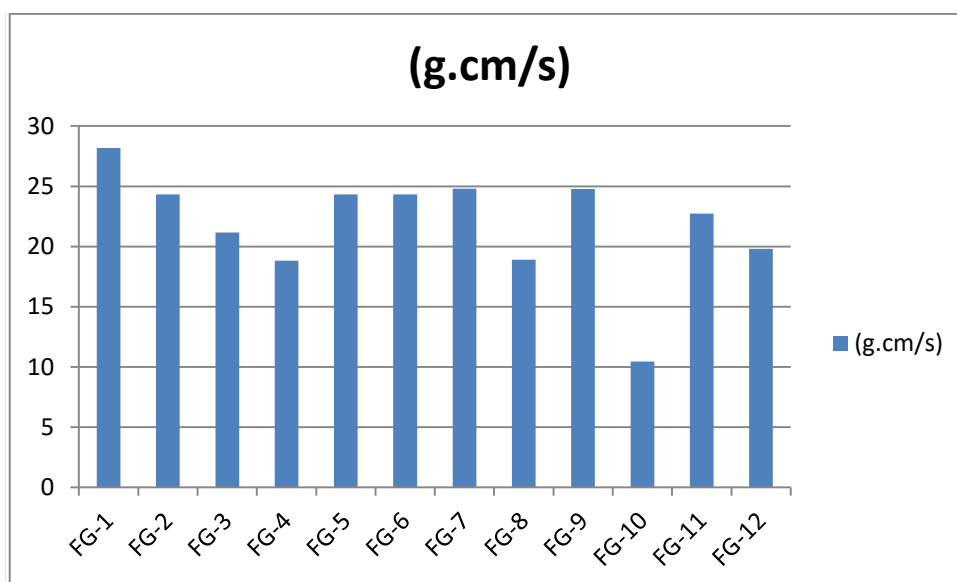


Fig 5: Spreadability of Fenugreek Cream

Discussion: Spreadability test was performed to check the spreadability of the cream when applied topically. With the help of this test, the smoothness of the cream and the spreading tendency of the cream was observed. Hence, with the help of this test, the texture of the cream be calculated which is stable for the cream stability. By performing this test it was found that FG-2,FG-5, FG-7, FG-8 and FG-10 were not able to pass this test (**shown in the table 4 and figure 5**)

Viscosity-The viscosity of the formulated batches was determined using a cone and plate viscometer with spindle 63 (Brookfield Engineering Laboratories). The assembly was connected to a thermostatically controlled circulating water bath maintained at 25 °C. The formulation whose viscosity was to be determined was added to a beaker covered with

thermostatic jacket. Spindle was allowed to move freely into the Cream. The spindle no.63 was dipped in Cream sample rotated a 10 rpm and $20 \pm 1^\circ\text{C}$ temperature for 15 min. The reading in triplicate was noted. Viscosity in centipoise (cp) was measured. Viscosity of the optimized Cream was found to be **64,500 cps**, which indicates that the Cream is neither too viscose nor too soft.

Viscosity of optimized cream

Table 5: Viscosity of Fenugreek cream Formulation.

Serial No.	Batch no.	Spindle no	Revolutions per minute	Viscosity (cp)
1	FG 1	S63	10rpm	449
2	FG2	S63	10rpm	418
3	FG 3	S63	10rpm	485
4	FG4	S63	10rpm	607
5	FG5	S63	10rpm	386
6	FG6	S63	10rpm	587
7	FG7	S63	10rpm	475
8	FG8	S63	10rpm	608
9	FG9	S63	10rpm	429
10	FG10	S63	10rpm	240
11	FG11	S63	10rpm	460
12	FG12	S63	10rpm	544

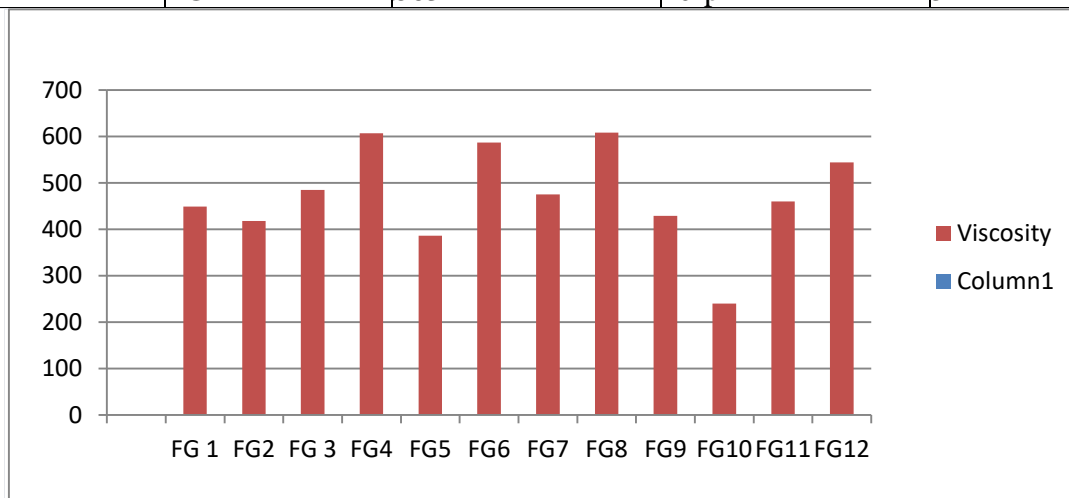


Fig 6: Viscosity of Fenugreek Cream

Discussion: By using Brookfield method, spindle no.63 was dipped in Cream sample rotated at 10 rpm and $20 \pm 1^\circ\text{C}$ temperature for 15 min. Best viscosity value was observed in FG-4 and FG-8 (Shown in Table 5 and fig 6). The value 65000cps indicates that the solution has stable viscosity. It is neither too viscous nor too soft. It is the perfect viscosity.

pH

1 % aq. a solution of Cream formulation was prepared and stored for 2 hours, pH was determined using a digital pH meter. The pH of each cream formulation was done in the triplicate, average value and \pm standard deviation was calculated.

The pH of the Cream formulations was in the range of 5 ± 0.2 to 7.5 ± 0.2 , which lies in the normal pH range of the skin and therefore it will not induce any skin irritation.

pH of Fenugreek Cream Formulation

Table 6: pH of Fenugreek Cream Formulation

S.no	Sample no.	pH
1	FG1	6.1
2	FG2	4.2
3	FG3	7.1
4	FG4	6.4
5	FG5	5.4
6	FG6	7.14
7	FG7	5.9
8	FG8	6.29
9	FG9	6.9
10	FG10	5.12
11	FG11	6.4
12	FG12	7.12

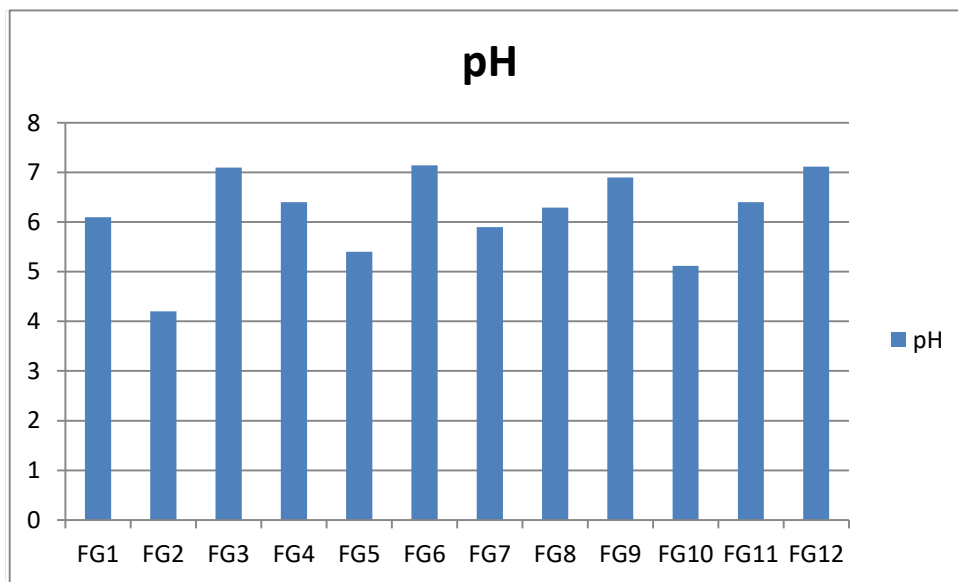


Fig 7: pH of Fenugreek Cream Formulation

Discussion: The pH data for fenugreek cream ranges from 5 ± 0.2 to 7.5 ± 0.2 , (as shown in table 6 and figure 7). The FG-2 shows low pH range 4.2 ± 0.2 which is not preferable for skin, Since it is strongly acidic may cause irritations, burning sensation when applied to the skin. It falls out of the limit. The favourable pH for skin 6.4 most preferable s observed on

formulation of FG-11, because pH can enhance the penetration of active ingredient through the stratum corneum, making formulation more effective. Lower pH can help in reducing skin irritation and sensitivity, making the formulation suitable for sensitive skin types. Lower pH environments can inhibit the growth of harmful bacteria and fungi, contributing to the preservation and safety of the product.

Homogeneity and Grittiness

All developed Cream formulations were allowed to set in a suitable container and tested for homogeneity. A small quantity of Cream was pressed between the thumb and the index finger. The consistency of the cream was noticed (whether homogenous or not), if there was any coarse particles appeared on fingers. Also, the homogeneity could be detected when a small quantity of the cream was rubbed on the back of the hand. The grittiness of the prepared cream was also observed in the same manner.

Table 7: Homogeneity of Fenugreek cream formulation

Serial No.	Batch no.	Homogeneity	Grittiness
1	FG 1	Homogenous	No
2	FG2	Not Homogenous	No
3	FG 3	Homogenous	No
4	FG4	Homogenous	No
5	FG5	Not Homogenous	No
6	FG6	Homogenous	No
7	FG7	Homogenous	No
8	FG8	Not Homogenous	No
9	FG9	Homogenous	No
10	FG10	Not Homogenous	No
11	FG11	Homogenous	No
12	FG12	Homogenous	No

Discussion: Homogeneity of the cream enhance the smoothness and uniform distribution of excipients and active drug in the cream formulation. homogeneity is checked at different temperature for number of days and this stability was observed the prepared formulation was stored for 2 to 6 weeks, for a temperature of $4 \pm 2^\circ\text{C}$ and $27 \pm 2^\circ\text{C}$, there were not differences of homogeneity, odor, texture except FG-5, FG-8 and FG-10(**as shown in Table 7**)

Assay Percentage (Drug content)

Method of analysis

Standard Solution-100mg of Fenugreek powder dissolved in 50 ml water boil it then make up volume 100ml with methanol. From the above solution 10 ml was pipette into a 100ml volumetric flask and made upto 100ml using methanol to get a stock solution of 100 $\mu\text{g}/\text{ml}$.

From this stock solution, a 10 ml was pipette and transfer into a 100ml volumetric flask and made up to mark with methanol to get a concentration in the range of 10 μ g/ml.

Test Solution-

1ml of Cream eq to 500mg of Fenugreek extract as dissolved in 100ml of methanol. From the above solution 2 ml was pipette out into a 100ml volumetric flask and made upto 100ml using methanol to get a stock solution of 100 μ g/ml. From this stock solution, a aliquots of 10 ml were pipette out into a 100ml volumetric flask and made up to mark with methanol to get a concentration in the range of 10 μ g/ml. The absorbance of the resulting solution was then measured at 295.5nm using UV Double beam spectrophotometer against methanol as blank. using UV-spectrophotometer.

Drug content of all samples is observed and calculated.

Table 8: Drug content in the Formulation prepared(Fenugreek Cream)

Serial no.	Batch no.	Drug content
1	FG 1	81.88%
2	FG2	88.54%
3	FG 3	96.72%
4	FG4	92.22%
5	FG5	87.14%
6	FG6	90.5%
7	FG7	96.37%
8	FG8	98.23%
9	FG9	90.36%
10	FG10	98.32%
11	FG11	99.11%
12	FG12	94.65%

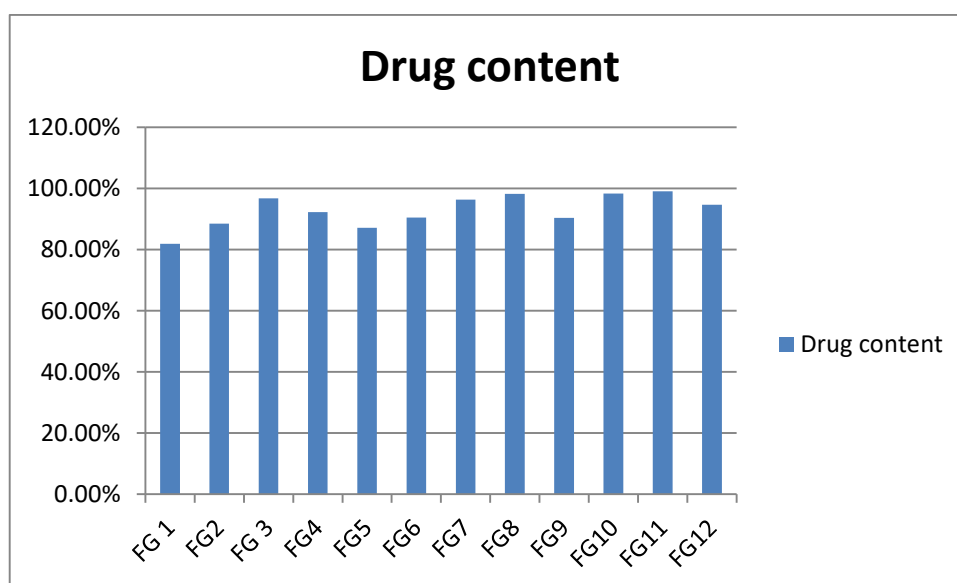


Fig 8: Drug content in various samples of Fenugreek Cream Formulation.

Discussion: Drug content in various samples found to be in limits as per Assay (**shown in table 8 and figure 8**).). The FG1, FG2, FG5, FG9 and FG3 shows low drug content there is many factors leading to low drug contents, like suboptimal formulation process (inefficient mixing, encapsulation or stabilization) can lead to lower drug content. Larger particles or unevenly distributed particles may have smaller surface area for drug encapsulation, resulting in lower drug content. In contrast, the FG11 shows high drug content due to optimized formulation process include efficient formulation techniques, such as proper mixing , encapsulation can enhances the penetration of drug content. Smaller and uniformly distributed particles can provide a larger surface area for drug absorption, increasing drug content. Thus, the best result shown in **FG11** with its higher drug content **99.11%** is more suitable for cream formulations. Higher drug content ensures greater efficacy, consistent dosing, and cost effectiveness. The formulation process, particle size, and drug – excipients compatibility play critical role in achieving high drug content in cream formulations. And also shows that good capacity of formulation to hold the drug.

Result

Evaluation of Topical cream for pH, Spreadability and Homogeneity and Grittiness and drug content.

Table 9: Evaluation of Topical cream for pH, Spreadability and Homogeneity and Grittiness and drug content

Batch no.	pH	Viscosity (cp)	Spreadability	Homogeneity	Grittiness	Drug content (%)
			(g.cm/s)			
FG 1	6.1	449	28.17	Homogenous	No	81.88%
FG2	4.2	418	24.32	Not Homogenous	No	88.54%
FG 3	7.1	485	21.15	Homogenous	No	96.72%
FG4	6.4	607	18.81	Homogenous	No	92.22%
FG5	5.4	386	24.32	Not Homogenous	No	87.14%
FG6	7.14	587	24.325	Homogenous	No	90.5%
FG7	5.9	475	24.81	Homogenous	No	96.37%
FG8	6.29	608	18.91	Not Homogenous	No	98.23%
FG9	6.9	429	24.78	Homogenous	No	90.36%
FG10	5.12	240	10.44	Not Homogenous	No	98.32%
FG11	6.4	460	22.74	Homogenous	No	99.11%
FG12	7.12	544	19.79	Homogenous	No	94.65%

Discussion:

Based on these results, it can be inferred that the Fenugreek extract Cream formulation no FG-2 pH fall to 4.2 which is out of range and not homogeneous and FG-5 and FG-7 is not homogeneous and FG-10 is also not homogeneous and its viscosity is poor all other formulations show good appearance and passes all the tests within limits . Thus, we can say that this all other formulation of fenugreek except FG-2, FG-5 and FG-7, FG-8, FG-9, FG-10 are not upto the mark FG-4, FG-11 and FG-12 are the formulations will give satisfactory affects. After achieving the formulation preparation, the next step is to check the stability of the product formed. The stability process is major part of preparation because the cream will

be successfully formulated only when it is stable for long period of time. We will check the stability of cream by performing stability test for three months.

IN –vitro Drug Release Studies of Fenugreek cream formulations

IN-vitro drug release studies revealed that the release of Fenugreek from different formulations varies with characteristics and composition of excipients. The comparison of the drug release profile of all formulations showed that formulation GF-11 shows maximum drug release of 99.11%. And all the others release studies of formulations showed in Table no.10

In Vitro drug release study

Table 10: In Vitro drug release study

Time (hr)	Cumulative Percentage of drug release											
	FG1	FG2	FG3	FG4	FG5	FG6	FG7	FG8	FG9	FG10	FG11	FG12
0.5	19.21 ±0.09	19.56 ±0.09	19.28 ±0.09	18.32 ±0.09	19.73 ±0.09	19.48 ±0.09	19.48 ±0.09	19.48 ±0.09	19.48 ±0.09	19.48 ±0.09	20.61 ±0.09	19.21 ±0.09
1	32.40 ±0.08	36.17 ±0.08	33.98 ±0.08	39.66 ±0.08	34.04 ±0.08	30.75 ±0.08	34.04 ±0.08	40.15 ±0.08	34.04 ±0.08	37.65 ±0.08	44.13 ±0.08	38.45 ±0.08
2	45.21 ±0.07 1	45.27 ±0.071 1	49.91 ±0.07 1	48.98 ±0.07 1	43.17 ±0.07 1	49.67 ±0.07 1	45.44 ±0.07 1	45.67 ±0.071 1	51.89 ±0.07 1	48.98 ±0.07 1	52.59 ±0.07 1	50.47 ±0.07 1
4	64.06 ±0.08 1	62.08 ±0.081 1	61.66 ±0.08 1	69.98 ±0.08 1	59.27 ±0.08 1	62.23 ±0.08 1	66.12 ±0.08 1	64.39 ±0.081 1	69.11 ±0.08 1	65.45 ±0.08 1	69.96 ±0.08 1	62.76 ±0.08 1
6	69.81 ±0.05 1	69.14 ±0.051 1	69.67 ±0.05 1	73.95 ±0.05 1	75.71 ±0.05 1	64.67 ±0.05 1	75.43 ±0.05 1	71.87 ±0.051 1	75.26 ±0.05 1	74.21 ±0.05 1	76.37 ±0.05 1	70.67 ±0.05 1
8	82.96 ±0.08 7	82.54 ±0.087 7	87.99 ±0.08 7	87.91 ±0.08 7	81.88 ±0.08 7	89.19 ±0.08 7	87.98 ±0.08 7	84.78 ±0.087 7	89.98 ±0.08 7	84.78 ±0.08 7	89.98 ±0.08 7	88.78 ±0.08 7
10	96.78 ±0.09 1	89.97 ±0.091 1	87.28 ±0.09 1	93.45 ±0.09 1	84.66 ±0.09 1	87.56 ±0.09 1	94.24 ±0.08 7	96.78 ±0.087 7	98.71 ±0.08 7	97.56 ±0.08 7	98.97 ±0.09 1	82.56 ±0.09 1

Mean ± SD; n = 3

Discussion: From the table and figure. The in vitro drug release pattern of initial burst release of surface adsorbed drug was observed followed by the initial burst effect on the surface release of Fenugreek may be due to the loosely associated fenugreek on the surface. The burst release is clinically significant to achieve initial high drug concentration in the target tissue. The slow release of the drug is controlled by the speed of the degradation of polymer. During

the drug release study the reservoir condition was maintained by regularly replacing the dialysis medium. The two phase drug release drugs behavior might be responsible that the initial rapid drug release is due to the release drugs weakly networked with hydrophobic moiety and the following steady release is due to the release of drug robustly interacted with hydrophobic core.

Stability Study of the Cream

After completing the pre formulation studies, stability studies are taken into consideration. Since, Im preparing the formation of fenugreek extract cream , in which I have taken 12 samples and tests are performed on theses samples. After short listing the samples which are not upto the mark, moving forward for stability testing.

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of environmental factors such as temperature, humidity and light. The stability of developed Cream was established over a period of 90 days' time intervals, the optimized formulation was kept at $40^{\circ}\text{C} \pm 2/\text{RH}70\% \pm 5$ and was evaluated for organoleptic properties, pH and drug content and the results are mentioned in the Table 11. Results indicates that the formulation was stable and no significant changes were observed

Stability data for Fenugreek Cream formulation.

Table11: Stability data for Fenugreek Cream formulation

S.NO	Color change	Creaking	Phase Separation	pH	Drug Content (%)
FG 1	No colour change	Observed	Observed	6.5	97.59
FG2	Slight change Observed	Observed	Observed	3.3	90.83
FG 3	Slight colour change	Not Observed	Not Observed	4.4	95.26
FG4	Not change Observed	Not Observed	Phase Separation	6.7	95.93
FG5	Slight colour change	Observed	Not Observed	6.39	96.95
FG6	Not change Observed	Not Observed	Not Observed	7.1	98.41
FG7	Not change Observed	Not Observed	Not Observed	6.9	98.90
FG8	Not change Observed	Observed	Observed	6.28	95.98
FG9	Not change Observed	Not observed	Not Observed	6.33	97.52
FG10	Not change Observed	Observed	Observed	6.28	96.41

FG11	Not change Observed	Not Observed	Not Observed	7.1	98.44
FG12	Slight colour change	Observed	Observed	5.9	95.92

Conclusions

While natural aging is determined genetically and extrinsic aging can also be prevented. Aesthetic dermatology playing a significant part in prevention, regeneration, and delaying of skin aging and also should contribute to “healthy aging” not only in cosmetic means by trying to erase time vestiges in skin with combining knowledge of possible local and systemic therapy, instrumental devices and invasive procedures, filling the lack of scientific investigations and becoming one of the important focuses of the aging research.

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