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Research Article

Formulation and Evaluation of Herbal Face Serum for Hyperpigmentation

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ABSTRACT

Face serums are widely used in skincare to address concerns such as wrinkles, acne, dryness, and dull-looking skin. These formulations improve skin appearance by providing hydration, enhancing brightness, and delivering targeted benefits. A key advantage of serums is their ability to absorb quickly and penetrate deeper layers of the skin, allowing active ingredients to act more effectively. The present study aimed to formulate and evaluate a safe and effective herbal face serum using natural ingredients including Aloe vera, liquorice root extract (Glycyrrhiza glabra), papaya leaf extract (Carica papaya), sesame oil, and lemon oil for the management of hyperpigmentation. The formulated serum was evaluated for physicochemical parameters including pH, viscosity, spreadability, homogeneity, skin irritation, washability, after feel, and stability. Three formulations (F1, F2, F3) were prepared and compared. Formulation F1 showed optimal results with a pH of 5.5–5.8, excellent viscosity (1518 cP), good spreadability, uniform homogeneity, non-irritant nature, and high stability. A tyrosine kinase inhibition assay demonstrated that the herbal serum exhibited concentration-dependent anti-tyrosinase activity with an IC50 of 93.40 µg/mL. The formulation was found to be safe, stable, and effective as a natural alternative for reducing hyperpigmentation and improving overall skin appearance.

INTRODUCTION

The term cosmetics originates from the Greek word kosmetikos, meaning 'to adorn.' Cosmetology is the scientific study and application of cosmetic products to enhance the appearance of skin, hair, and nails. Serums are highly concentrated formulations, either water-based or oil-based, designed to deliver active ingredients effectively into the skin. Due to their low molecular weight and viscosity, they penetrate deeper and act faster than conventional creams.[1,2,3]

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The skin, also known as the integumentary system, is the largest organ of the human body and serves as the first protective barrier against microbes, chemicals, and physical injury. It is composed of three main layers: epidermis, dermis, and subcutaneous tissue, each having specific functions in protection and maintenance of the body.[4,5]

Skin pigmentation is determined by melanin produced by melanocytes in the basal layer of the epidermis through the process of melanogenesis, involving the enzyme tyrosinase. Hyperpigmentation is the darkening of certain skin areas due to increased melanin production caused by sun exposure, hormonal changes, inflammation, or aging. Herbal and natural cosmetic products are increasingly preferred for pigmentation management because they are considered safer, effective, and associated with fewer side effects.[8,9,10,11]

Hyperpigmentation occurs due to increased melanin synthesis or its uneven distribution, influenced by both internal and external factors such as UV radiation, hormonal changes, post-inflammatory responses, aging, genetic factors, medications, and nutritional disorders.[12,13,14] The present work was undertaken to develop a safe and effective herbal face serum incorporating plant-based extracts for the management of hyperpigmentation with minimal side effects.

Materials and Methods

Materials Used

- Aloe vera gel
- Lemon Oil
- Sesame Oil
- Glycerine
- Tween 80
- Methyl Paraben
- Rose Oil / Perfume

Instruments Used

- Electric Balance
- Digital pH-meter
- Sonicator / Brookfield Viscometer

Collection and Authentication of Plant Material

Liquorice (Glycyrrhiza glabra): The roots of Glycyrrhiza glabra were collected from the herbal garden of Dr. J. J. Magdum Pharmacy College, Jaysingpur. The roots were dried in shade to preserve active constituents, then crushed and powdered using an electrical grinder. The plant material was authenticated by the Department of Botany, Jaysingpur College, Jaysingpur (Date: 02/04/2026).[34,35]

Papaya Leaf (Carica papaya): The leaves of Carica papaya were collected from Jaysingpur, Tal-Shirol, Dist-Kolhapur, Maharashtra, India. The plant material was dried in shade, ground into a powder using an electrical grinder, and authenticated by the Department of Botany, Jaysingpur College, Jaysingpur (Date: 02/04/2026).[36,37]

Extraction

Papaya Leaf Extract: About 10 g of dried papaya leaf powder was soaked in 100 ml of 70% ethanol. The flask was tightly closed and kept at room temperature for 72 hours with occasional shaking. After extraction, the solution was filtered using

Whatman No.1 filter paper to obtain the papaya leaf extract.[41,42]

Liquorice Extract: About 10 g of dried liquorice root powder was soaked in 100 ml of 70% ethanol. The mixture was kept at room temperature for 72 hours with occasional shaking. After extraction, the solution was filtered through Whatman No.1 filter paper to obtain the liquorice extract.[43,44]

Phytochemical Screening

Phytochemical screening of both plant extracts was carried out using standard methods.[38,39,40] Glycyrrhiza glabra extract was positive for saponins (Foam test and Libermann Burchard test), flavonoids (Shinoda test and Lead acetate test), and terpenoids (Salkowski reaction). Papaya leaf extract was positive for alkaloids (Dragendorff's and Wagner's tests), glycosides (Keller-Killiani, Baljet, and Legal's tests), tannins (Lead acetate test), flavonoids (Shinoda test and Lead acetate test), and saponins (Foam test).

Formulation of Herbal Face Serum

Three formulations (F1, F2, F3) were prepared using varying concentrations of herbal extracts as shown in Table 3.

Table 3: Formulation of Herbal Face Serum (Total Volume 15 ml)

Ingredient	F1	F2	F3
Aloe vera gel	9.75 ml	9.00 ml	8.25 ml
Papaya leaf extract	1.50 ml	2.25 ml	2.75 ml
Liquorice extract	2.25 ml	2.25 ml	2.50 ml
Lemon oil	0.30 ml	0.30 ml	0.30 ml
Sesame oil	0.37 ml	0.37 ml	0.50 ml
Glycerin	0.45 ml	0.45 ml	0.50 ml
Tween 80	0.30 ml	0.30 ml	0.30 ml
Methyl Paraben	0.02 mg	0.02 mg	0.05 mg
Perfume	q.s.	q.s.	q.s.

Preparation Procedure

Step 1 – Oil Phase: Lemon oil, sesame oil, and Tween 80 were mixed in a clean dry beaker with continuous stirring for 10 minutes to obtain a uniform oily mixture.

Step 2 – Aqueous Phase: Aloe vera gel, papaya leaf extract, liquorice extract, glycerine, and methyl paraben was mixed thoroughly. Distilled water was added as needed to obtain a smooth uniform mixture.

Step 3 – Emulsion Formation: The oil phase was added dropwise into the aqueous phase with continuous stirring and trituration to form a stable oil-in-water (O/W) emulsion.

Step 4 – Perfume Addition: A suitable quantity of perfume was added and stirred gently for even distribution.

Step 5 – Homogenization: The serum was mixed until smooth and homogeneous.

Step 6 – Filling and Storage: The prepared serum was transferred into a clean airtight container and

stored at room temperature away from direct sunlight.[48,49,50]

Evaluation Parameters

The formulations were evaluated for the following parameters:[52,53,54,55,56]

- Physical Appearance – colour, odour, and texture were assessed visually and by touch.
- pH – measured using a digital pH meter; acceptable range 4.5–6.5.
- Viscosity – determined using a Brookfield Viscometer.
- Spreadability – evaluated using glass slide method with 50 g weight for 1 minute.
- Homogeneity – assessed by visual observation on a glass slide.
- Skin Irritation – patch test applied for 24 hours; absence of redness or itching indicates safety.
- Washability – ease of removal with water assessed.
- After Feel – evaluated by applying serum and assessing skin feel.
- Stability Study – stored under different conditions; observed for colour, odour, texture, pH, and phase separation changes.

Tyrosine Kinase Inhibition Assay

The tyrosine kinase inhibitory activity of the test sample was evaluated by an in-vitro assay using 50 mM Tris-HCl buffer (pH 7.5) containing MgCl2 and DTT. Different concentrations of the test sample and standard drug Imatinib Mesylate were added to labeled microplate wells followed by tyrosine kinase enzyme. After pre-incubation at 37°C, ATP and poly-Glu:Tyr peptide substrate were added to initiate the reaction. Absorbance was measured at 405/450 nm and percentage inhibition and IC50 values were calculated. % Inhibition = [(C – T) / C] × 100, where C = absorbance of control and T = absorbance of test sample.

Results and Discussion

Three herbal face serum formulations (F1, F2, F3) were successfully prepared and subjected to various evaluation tests. The results are summarized below.

Table 4: Physical Examination of Formulations

Sr. No.	Physical Parameter	F1	F2	F3
1	**Colour**	Light yellowish brown	Yellowish brown	Dark brownish
2	**Odour**	Pleasant	Pleasant	Pleasant
3	**Texture**	Smooth and non-greasy	Slightly tacky/sticky	Thin and non-uniform

Table 5: Evaluation of Formulations

Sr. No.	Evaluation Test	F1	F2	F3
1	**pH**	5.5–5.8	5.8–6.0	6.1–6.2
2	**Viscosity**	1518 cP (Excellent)	1502 cP (Moderate)	1486 cP (Slightly watery)

3	**Spreadability**	Excellent (easy to apply)	Good	Fair (uneven)
4	**Homogeneity**	Uniform, no lumps	Uniform	Slight lumps noticed
5	**Skin Irritation**	Non-irritant	Non-irritant	Non-irritant
6	**Washability**	Easy to wash	Easy to wash	Easy to wash
7	**After Feel**	Non-sticky, smooth	Non-sticky	Slightly sticky/oily
8	**Stability**	Highly stable	Stable	Slight sedimentation observed

Table 6: Tyrosine Kinase Inhibition Assay Results

Sample	IC50 (µg/mL)	Observation
Standard (Imatinib Mesylate)	71.28	Showed highest tyrosine kinase inhibitory activity
Herbal Face Serum (F1)	93.40	Exhibited significant tyrosine kinase inhibitory activity indicating potential for anti-hyperpigmentation

Formulation F1 demonstrated the best overall performance among the three formulations. Its pH (5.5–5.8) was within the acceptable skin-compatible rangeof 4.5–6.5, ensuring no irritation on topical application. The excellent viscosity of 1518 cP provided a suitable consistency for a serum formulation, while the good spreadability allowed easy and uniform application on the skin surface. The formulation was found to be homogeneous with no lumps, non-irritant on patch testing, easily washable, and imparted a non-sticky smooth after feel. Stability studies revealed no phase separation, colour change, or physical instability under different storage conditions.

The tyrosine kinase inhibition assay showed that the herbal face serum exhibited concentration-dependent inhibitory activity with an IC50 of 93.40 µg/mL, compared to 71.28 µg/mL for the standard Imatinib Mesylate. Although the standard showed higher inhibition, the formulation demonstrated promising anti-tyrosinase activity, confirming its potential for skin brightening and reducing hyperpigmentation.

Aloe vera contributed excellent moisturizing and soothing properties due to its rich content of vitamins and minerals. Liquorice extract, containing glabridin and glycyrrhizin, provided anti-hyperpigmentation and skin-brightening effects by inhibiting tyrosinase activity. Sesame oil, rich in vitamin E and antioxidants, protected the skin from UV and environmental damage. Lemon oil contributed antimicrobial and antioxidant activities. Papaya leaf extract, containing papain and flavonoids, provided exfoliating and anti-inflammatory effects. These findings are in agreement with earlier reports on herbal serums.1,3,8,25,26

CONCLUSION

The present study successfully formulated and evaluated an herbal face serum using natural ingredients for the management of hyperpigmentation. Formulation F1, containing 9.75 ml aloe vera gel, 1.50 ml papaya leaf extract, and 2.25 ml liquorice extract as the principal active components, demonstrated optimal physicochemical properties including a skin-compatible pH, excellent viscosity, good spreadability, uniform texture, and high stability. All herbal components were successfully incorporated without affecting the appearance, texture, or stability of the formulation. The tyrosine kinase inhibition assay confirmed the anti-hyperpigmentation potential of the serum. The formulated herbal face serum represents a safe, effective, and natural alternative to synthetic skincare preparations. Further clinical studies and long-term evaluations on a larger scale are recommended to confirm its effectiveness and safety.

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