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AN ANTI PIGMENTATION STICK- THE NOVEL AND INNOVATIVE FORMULATION

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Abstract

Hyperpigmentation is a common dermatological concern caused by excessive melanin production due to factors such as sun exposure, hormonal imbalance, inflammation, and aging. The present study aimed to formulate and evaluate an anti-pigmentation herbal stick using natural and synthetic depigmenting agents for convenient topical application and improved patient compliance. The formulation was developed using licorice root extract (Glycyrrhiza glabra) and kojic acid as the primary active ingredients because of their well-known skin-brightening and melanin-inhibiting properties. The anti-pigmentation stick was prepared using beeswax and stearic acid as structuring agents, while aloe butter and shea butter were incorporated to provide moisturization and skin nourishment. MCT oil and almond oil were used as emollients to enhance spreadability and improve skin conditioning. Arrowroot powder was added to impart a smooth texture and reduce greasiness. Vitamin E oil served as an antioxidant to protect the formulation from oxidative degradation, and phenoxyethanol was used as a preservative to ensure microbial stability.

INTRODUCTION:

Pigmentation [1]:

Skin pigmentation refers to the natural color of human skin, which is mainly determined by a pigment called Melanin. Melanin is produced by melanocytes through a process called melanogenesis. Melanin is produced by specialized cells known as melanocytes, present in the basal layer of the Epidermis.

Types of Melanin:

1. **Eumelanin:**
   The dark pigment known as eumelanin has a brown to black appearance. In human skin, hair, and eyes, it is the most common form of melanin.
2. **Pheomelanin:**
   Pheomelanin is a yellow to reddish pigment. It occurs most commonly in people with light skin, blonde hair, or red hair.

Types of Pigmentation:

Skin color disorders arise due to abnormality in melanin synthesis. Skin color disorders are mostly categorized into:

Hyperpigmentation: This is a condition characterized by excessive production of

melanin resulting in darker areas on the skin.

Common Forms of Hyperpigmentation: Melasma Post-inflammatory hyperpigmentation(PIH) Solar Lentigines.

Hypopigmentation: Hypopigmentation is defined as the condition characterized by low production or absence of melanin pigments resulting in lighter patches on the skin.

Common Forms of Hypopigmentation: Vitiligo – autoimmune destruction of melanocytes Albinism – genetic reduction in melanin production Pityriasis alba – patchy white spots usually found in children

Role of Melanin in Pigmentation:

Melanin plays several important biological roles:

Photoprotection: It protects skin cells from UV-induced DNA damage and reduces the risk of skin cancers.

Antioxidant activity: Melanin helps neutralize reactive oxygen species generated by UV exposure.

Thermoregulation and adaptation: Changes in pigmentation have been developed as adaptations to varying levels of UV radiation in different environments.

Melanin production rises following exposure to sunlight, and this phenomenon is referred to as facultative pigmentation or tanning. This adaptive response evolved as humans migrated to regions with different UV exposure levels. Thus, melanin is not only responsible for visible skin color but also serves as an evolutionary and protective mechanism essential for human survival. When sunlight exposure increases, it can harm the skin barrier, resulting in increased melanin production activated, leading to skin darkening.

Formation of Melanosomes: [3]

Melanosomes originates from the endosomal compartment of melanocytes. They are specialized organelles in melanocytes where melanin is synthesized, stored and later transferred to keratinocytes.

Melanosomes develop through four maturation stages (I – IV): Pre melanosome formation, Fibril Formation, Initiation of melanisation and Mature melanosomes.

Factors regulating Melanogenesis[4]:

Genetic Factors Enzymatic Factors Hormonal Factors Environmental Factors[5]

Topical drug delivery:

Oral, sublingual, rectal, parenteral, topical, and inhalation routes have all been developed in recent years as ways to administer medications to the body. Applying a medication formulation directly to the skin is known as topical drug delivery, and it is used to treat skin disorders including psoriasis.

Topical drug delivery systems are mainly designed to:

- Deliver the drug directly to the affected skin area
- Improve patient comfort and ease of application
- Enhance therapeutic effectiveness with fewer systemic side effects
- Maintain prolonged drug action at the site of application

Advantages of topical drug delivery:

- Direct drug delivery to the target site
- Reduced systemic side effects
- Avoidance of first-pass metabolism
- Improved patient compliance
- Sustained and localized action
- Better cosmetic acceptability
- Ease of termination of therapy
- Suitable for various dermatological conditions

Anti – Pigmentation Stick:

Anti-pigmentation sticks are solid topical cosmetic/pharmaceutical preparations designed to reduce and prevent skin hyperpigmentation such as dark spots, melasma, acne marks, and uneven skin tone. They contain active ingredients that help inhibit melanin production, reduce

tyrosinase activity, promote skin renewal, and protect skin from UV-induced pigmentation. These sticks are applied directly to the affected area and are preferred for their convenience, portability, precise application, non-greasy nature, and ease of use. Common active agents used in anti-pigmentation sticks include Kojic Acid, Ascorbic Acid, Azelaic acid, Niacinamide, and alpha arbutin.

MATERIALS AND METHODS

Plant material

Licorice roots (*Glycyrrhiza glabra Linn*.) were procured from a certified herbal supplier in Maharashtra, India, during January 2026

The roots were authenticated by a qualified botanist at the Department of Botany, Radhabai Kale Mahila Mahavidyalaya, Ahilyanagar.

Kojic acid was procured from a certified pharmaceutical chemical supplier and was of analytical grade. The material was stored in a well-closed container protected from light and moisture until use. Kojic acid was incorporated into the formulation as a depigmenting agent due to its tyrosinase inhibitory activity.

Preparation of Licorice Root Extract[6]

Licorice root powder (20 g) was extracted by the maceration method using a hydroalcoholic solvent consisting of ethanol (70 mL) and water (30 mL). The roots were thoroughly washed, dried, and powdered before extraction. The mixture was kept at room temperature for 72 hours with occasional stirring to facilitate extraction.

After the maceration period, the extract was filtered through muslin cloth to separate the liquid extract from the residue. The obtained filtrate was then stored in a cool and dry place for further use in the formulation.

Identification tests for licorice root extract

The prepared hydroalcoholic extract of licorice root was subjected to preliminary phytochemical screening for the identification of major phytoconstituents.

The results of phytochemical screening confirmed the presence of flavonoids[7], glycosides[8], tannins[9], and saponins[10] in the licorice root extract, which may contribute to its anti-pigmentation and antioxidant activities. Alkaloids[11] were not detected in the extract.

Table No. 1 – Phytochemical screening

Sr. No.	Phytoconstituent	Test	Observation	Inference
1.	**Flavonoids**	**Alkaline reagent test** :1 ml extract + few drops of NaOH solution + dilute acid	Intense yellow color which disappeared after adding dilute acid	**Present**
		Lead acetate test :1 ml extract + few drops of lead acetate solution	Formation of yellow precipitate	
		Ferric chloride test :1 ml extract + Ferric chloride solution	Dark green color appeared	

2.	**Glycosides**	**Killer–Killani test**: 1 ml extract + 1 ml ferric chloride + 1ml concentrated sulfuric acid	Brown ring appeared at the junction of the test tube	**Present**
		Foam test : Add extract to water and shake vigorously	Stable foam that persisted for 10-15 minutes	
3.	**Alkaloids**	**Dragendorff's reagent test** : Take 2 ml acidic extract of licorice + few drops of Dragendorff's reagent	No formation of orange brown precipitate	**Absent**
		Hager's reagent test :1 ml extract + 1 ml picric acid solution + 1 ml hager's reagent	No formation of yellow precipitate	
4.	**Tannins**	**Ferric chloride test** :1 ml extract + 2 drops of ferric chloride solution	Blue color appeared	**Present**
		Lead acetate test :1 ml extract + few drops of lead acetate solution	Formation of yellow precipitate	
5.	**Saponins**	**Foam test** :1 ml extract + 5 ml of distilled water and shake vigorously	Stable foam that persisted for 10-15 minutes	**Present**
		Lead acetate test **:1 ml extract + lead acetate solution**	**Formation of white precipitate**	

Method of preparation:
Preparation of anti – pigmentation stick:
Step I: Melting
Melt beeswax, shea butter, aloe butter and stearic acid using water bath (70-75^{0}C)
Step II: Adding Oils
Add almond oil, Vitamin E oil, and MCT oil to the melted wax and butters.
Step III: Cooling
Cool this mixture at 40^{0}C.
Step IV: Adding Actives
Licorice extract, kojic acid and sandalwood powder, and keep stirring slowly to avoid lumps.
Step V: Adding Preservatives
Add arrowroot powder and phenoxyethanol and continue stirring.
Step VI: Molding
Now pour the mixture into stick molds and allow it to solidify at room temperature.

To set the Formula

Table No. 2 – Formulation table[12]

Sr. no.	Ingredients	F1	F2	F3	F4	Role
1	**Licorice extract (gm)**	0.7	1	1	1	**Natural skin lightening agent**
2	**Kojic acid (gm)**	0.5	0.4	1	1	**Depigmenting agent**
3	**Sandalwood powder (gm)**	0.3	0.5	0.5	0.5	**Enhances fragrance**
4	**Beeswax (gm)**	2.5	2.18	2	2	**Structuring agent**
5	**Shea butter (gm)**	2.5	2.5	1	0.8	**Emollient**
6	**Aloe butter (gm)**	0.5	0.6	1	1.10	**Provides moisturizing effects**
7	**Vitamin E oil (ml)**	0.1	0.05	0.03	0.04	**Antioxidant**
8	**MCT Oil (ml)**	0.3	0.3	0.12	0.3	**Enhances spreadability**
9	**Almond oil (ml)**	0.5	0.4	0.3	0.3	**Skin softening agent**
10	**Arrowroot powder (gm)**	1.2	1.1	1.4	1.26	**Absorbent**
11	**Stearic acid (gm)**	0.8	0.9	1.3	1.4	**Hardening agent**
12	Phenoxyethanol (ml)	**0.1**	**0.07**	**0.35**	**0.3**	**Preservative**

Anti – Pigmentation Stick

Fig. No. 1 – Anti – pigmentation sticks (batch F1 – F4)

Evaluation Parameters[12]

1. **Organoleptic evaluation:**
   It refers to the evaluation of anti – pigmentation stick based on the parameters such as color, odor, texture, and homogeneity
2. **Irritancy test:**
   The formulated stick was applied to skin and observed the effects. No irritation, redness, or swelling occurred after application. And hence the formulation is non – irritant.
3. **Stability study:**
   Stability of the formulated stick was determined by keeping it under different temperatures. And it was found to be stable.
4. **Determination of pH**:

1 gm of stick was dispersed in 10 ml of distilled water. The electrode of digital pH meter was dipped in this dispersion to calculate the pH..

5. **Melting point:**
   For calculating melting point, a small sample of formulation was heated in a heating pan. Temperature of the sample was measured using a thermometer. T1 is the temperature when it started melting and T2 is the temperature when it completely melted.
   Finally, melting point was calculated using formula:
   M.P = (T1 + T2) / 2
6. **Solubility:**
   Insoluble in water, dispersed in oils and heat
7. **Spreadability:**
   To determine the spreadability, a small sample of the formulation was placed between two glass slides and weight was applied to the upper slide. Time required to separate two slides was measured. Finally, spreadability was calculated using the formula:
   S = M x L / T
8. **In-vitro evaluation of anti – pigmentation stick**
   Tyrosinase activity inhibition was measured using a Microplate Reader. Tyrosinase enzyme solutions, LDOPA, and test samples were all dissolved in a 20 mmol pH 6.8 phosphate buffer solution. Mushroom tyrosinase activity was measured in 96-well plates. 50 µl of a test sample solution, 150 µl of 20 mmol phosphate buffer at pH 6.8, and 50 µl of mushroom tyrosinase (100 unit/ml, E. C. 1.14.18.1, Sigma) made up the reaction mixture. For ten minutes, the mixture was pre-incubated at 25 °C. The mixture was then incubated for 60 minutes at 37 °C after 20 µl of 2.5 mmol L-DOPA was added. The color changed from colorless to orange when L-DOPA transformed into dopachrome during the process. The absorbance at 490 nm was used to measure this change. A microplate reader was used to measure the absorbance at 490 nm following a 60-minute incubation period at 37 °C. Using kojic acid as a positive control, the tyrosinase inhibitory percentages were computed at a concentration of 120 µg/ml. Tyrosinase inhibitory activity percentages are computed using the following formula:

   Inhibitory activity of tyrosinase = (A0 – A1 / A1) x 100
   where A0 is the control solution's absorbance and A1 is the sample's absorbance.

RESULT AND DISCUSSION

Table No. 3 – Evaluation of anti – pigmentation stick

Parameters	F1	F2	F3	F4
Color	Yellowish brown	Brown	Brown	**Light brown**
Odor	Unnoticeable	Mild	Mild	**Mild**
Homogeneity	Granular	Granular	Granular	**Smooth**
Texture	Too oily	Oily	Hard	**Soft**
Irritability	No irritation	No irritation	No irritation	**No irritation**

Melting point	58.2	59.1	61.8	**63.4**
pH	6.2	5.9	5.6	**5.4**
Stability	Unstable	Stable	Unstable	**Stable**
Spreadability	**6.4**	**7.2**	**5.9**	**6.7**

Table No. 4 – % tyrosinase inhibition at a concentration of 120 µg/ml

Sr. no.	F1	F2	F3	F4	Kojic Acid
1	64.2	69.8	76.4	83.6	**96.8**
2	66.1	71.2	77.8	84.9	**97.5**
3	65.4	70.5	75.9	82.8	**97.1**
Average	65.23	70.50	76.70	83.77	**97.13**
Standard Deviation (SD)	0.96	0.70	0.98	1.06	**0.35**
Percentage Relative Standard Deviation (% RSD)	**1.47**	**0.99**	**1.28**	**1.27**	**0.36**

Tyrosinase inhibition activity of the anti-pigmentation stick formulations (F1–F4) was evaluated at a concentration of 120 µg/ml. The inhibitory activity increased progressively from F1 (65.23 ± 0.96%) to F4 (83.77 ± 1.06%), indicating enhanced anti-pigmentation potential with increasing concentration of active ingredients. Among the formulations, F4 exhibited the highest tyrosinase inhibitory activity. Pure Kojic acid, used as the positive control, showed 97.13 ± 0.35% inhibition, confirming the validity of the assay. The low %RSD values (<2%) for all samples indicated good reproducibility and precision of the experimental result.

CONCLUSION

The present study aimed to successfully formulate and evaluate an anti-pigmentation stick containing licorice root extract, kojic acid, sandalwood powder, beeswax, shea butter, aloe butter, almond oil, MCT oil, vitamin E oil, stearic acid, arrowroot powder, and phenoxyethanol. Four formulations (F1–F4) were prepared and subjected to various physicochemical and performance evaluations. Among all formulations, F4 demonstrated the most satisfactory characteristics, exhibiting good appearance, homogeneity, spreadability, stability, pH compatibility, and ease of application. The formulation showed no signs of phase separation,

irritation, or instability during the evaluation period. The incorporation of natural and synthetic depigmenting agents provided a synergistic effect, enhancing the overall performance of the formulation. The tyrosinase inhibition assay revealed that F4 possessed the highest anti-pigmentation activity among the tested formulations, indicating its potential to reduce melanin synthesis effectively. The presence of licorice root extract, rich in glabridin and flavonoids, along with kojic acid, contributed significantly to the inhibition of tyrosinase enzyme activity. Overall, the developed anti-pigmentation stick, particularly formulation F4, demonstrated promising efficacy, stability, and user acceptability. The results suggest that the formulation can serve as a convenient and effective topical drug delivery system for managing hyperpigmentation and improving skin complexion.

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