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Formulation and Evaluation of a Polyherbal Cream for the Management of Hyper Pigmentation

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ABSTRACT: This study focuses on the formulation and evaluation of a polyherbal cream containing Glycyrrhiza glabra (Licorice), Azadirachta indica (Neem), Curcuma longa (Turmeric), and Rubia cordifolia (Manjistha) for the management of hyperpigmentation. The herbal extracts were prepared by maceration and incorporated into an oil-in-water cream base. The formulated creams were evaluated for physicochemical parameters including pH, spreadability, washability, phase separation, and irritancy. Phytochemical screening confirmed the presence of active constituents such as flavonoids, tannins, and saponins. The results showed that the formulation possessed good stability, skin-friendly pH, non-irritant nature, and satisfactory application properties. The synergistic effect of these herbal ingredients indicates that the developed polyherbal cream can serve as a safe, effective, and natural alternative for reducing hyperpigmentation and improving overall skin complexion.

I. INTRODUCTION

Hyperpigmentation is one of the most common dermatological concerns affecting people of all ages and skin types. It refers to a condition in which certain areas of the skin appear darker than the surrounding tissue due to an overproduction or uneven distribution of melanin, the natural pigment responsible for determining skin, hair, and eye colour. This pigment is produced by specialized cells known as melanocytes, located in the basal layer of the epidermis. When these cells become overstimulated by sunlight, inflammation, hormonal changes, or other factors they produce excess melanin, which accumulates in specific regions of the skin, leading to visible dark patches or spots.

The intensity and appearance of hyperpigmentation can vary depending on the underlying cause, skin tone, and depth of pigment deposition. For some individuals, it may appear as faint freckles or sunspots, while others may develop large, irregular patches that are more noticeable.

Skin Pigmentation and Role of Melanin

The skin is the largest organ of the human body and serves as the primary protective barrier against environmental stressors such as ultraviolet (UV) radiation, pollutants, microorganisms, and chemical irritants. One of its most important physiological features is pigmentation, which determines skin color and plays a vital protective role.

Skin pigmentation is primarily governed by a pigment known as **melanin**, which is synthesized by specialized cells called melanocytes located in the basal layer of the epidermis. Melanin is produced within intracellular organelles called melanosomes and subsequently transferred to surrounding keratinocytes.

Melanin performs several critical functions:

- Protection against harmful UV radiation
- Neutralization of free radicals
- Prevention of DNA damage
- Determination of skin, hair, and eye color

Melanin synthesis (melanogenesis) occurs through the oxidation of the amino acid tyrosine in a pathway regulated by the enzyme tyrosinase. Any alteration in melanin production, distribution, or degradation can lead to pigmentation disorders. Thus, melanin is not only responsible for cosmetic appearance but also plays a crucial role in maintaining skin health.



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1.2 Herbs effective for treatment are:-

Licorice (*Glycyrrhiza glabra*) Licorice contains **glabridin**, which inhibits **tyrosinase enzyme**, the key enzyme in melanin synthesis. Useful for acne marks, irritation-induced pigmentation. Improves overall skin tone.

Neem (*Azadirachta indica*) possesses strong antimicrobial, anti-inflammatory, and immunomodulatory properties that help in maintaining skin health.

Turmeric (*Curcuma longa*) is well known for its antioxidant and anti-inflammatory effects due to the presence of curcumin, which may help reduce oxidative stress involved in vitiligo pathogenesis.

Manjistha (*Rubia cordifolia*) is widely used in Ayurveda as a blood purifier and skin rejuvenating herb and is believed to support healthy skin pigmentation.

The combination of these medicinal herbs in a polyherbal formulation may provide synergistic therapeutic effects for the management of vitiligo. Therefore, the present research aims to develop and evaluate a standardized polyherbal churna formulation containing Bakuchi, Neem, Turmeric, and Manjistha for its potential role in supporting repigmentation and improving skin health. Materials and Methods.

II. MATERIALS AND METHODS

The raw herbal drugs used in the formulation were, **Licorice (*Glycyrrhiza glabra*) root**, **Neem (*Azadirachta indica*) leaves**, **Turmeric (*Curcuma longa*) rhizome**, and **Manjistha (*Rubia cordifolia*) roots**. All the crude drugs were collected from a local herbal drug store and authenticated based on their morphological characteristics. The chemicals and reagents used for analytical testing were of analytical grade.

Plant Material

Fresh plant materials that is Leaves, stem and root are collected were taxonomically identified and authenticated by botanical expert. The plant materials were shade dried until all the water molecules evaporated and plants became well dried for grinding. Dry plant material separately grind to a fine powder and stored for further experiment with proper labels.

Preparation of Extracts

Dried powder of Leaves, Stem and Root powder for each experimental plant was exhaustively extracted successively by maceration extraction process. The solvents were removed by filtration process. The extracts were weighed and their percentage value was recorded and thereafter, was stored in refrigerator for further experimental work.

2.1 Phytochemical analysis

1) LICORICE

The extractions was tested for the presence of bioactive compounds by using following standard methods

Test for alkaloids: Hydroalcoholic Extract was mixed with few drops of Mayer's reagents. **Observation:** White ppt is the evidence for the presence of alkaloids.

Test for flavonoid: Take 1ml of hydroalcoholic extract, add few drops of concentrated HCL, then add few mg of magnesium. **Observation:-** Red or Orange colour shows the presence of flavonoids.

Test for tannins: Hydroalcoholic extract was mixed with 2ml of 1% solution of FeCl₃. **Observation:-** A blue-black coloration indicate the presence of tannins.

Test for phenolic: Hydroalcoholic extract was mixed with 2 ml of 2% solution of FeCl₃. **Observation:-** A blue-black color indicate the presence of Phenols.

Test for saponin: 1 ml of Hydroalcoholic extract was added with few drops of sodium bicarbonate solution then shake vigorously and left to stand for 5 min. **Observation:-** Foam indicate the presence of saponins.

2) NEEM

Test for alkaloids: Sample taken in test tube, added with 5ml HCl(1.5%) then filter it. Add few drops of Dragendroff's reagent. **Observation:-** Turbidity or any change

Test for carbohydrates: Take sample, add 1 ml Molisch reagent then heat on water bath for 2 minutes. **Observation:-** Green colour show sugar.

Test for tannin: Few ml of 0.1 % Ferric chloride taken with neem sample. **Observation:-** Blue-black colour.



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Test for saponins: Take 200 mg in 5 ml distilled water and shake. **Observation:-** Foam produced or not.
Test for flavonoids: Take few drops Sodium Hydroxide, add 1 ml sample. If it turns yellow then add dilute acetic acid.
Observation:- If yellow colour turns colourless, means flavonoids present.
Test for phenol: Take 1 ml sample solution, add 3 ml of ferric chloride (10%). **Observation:-** White precipitate indicate presence.



Fig.1 Identification test of Licorice



Fig.2 Identification test of Neem

3) MANJISTHA

Test for Alkaloids: Take sample and add Dragendroff's reagent. **Observation:** Orange colour ppt found.
Test for Carbohydrate: Take 1 ml Fehling's A and 1 ml Fehling's B with 1-2 ml of sample then boil. **Observation:** Brick-red ppt of reducing sugars.
Test for Saponin: 1 ml extract taken with 10 ml distilled water, shake for 15 mins. **Observation:** 1-1.5 cm of froth observed.
Test for Phenols: 2 ml drug sample solution added with 2 ml of ferric chloride solution. **Observation:** Blue-violet/Red or green colour shows presence.
Test for tannin: 1 ml extract with vanillin HCl reagent (vanillin 1 gm + alcohol 10 ml) **Observation:** Brick or red show presence.
Test for Flavonoids: Take 1 ml extract, add 5ml 95% ethanol and few drops pf concentrated HCl and 0.5 gm magnesium. **Observation:** Pink colour observation.

4) TURMERIC

Test for tannin:- 0.5 g of plant extract was mixed with 2mL of water and heated on water bath. The mixture was filtered and 1mL of 10% FeCl₃ solution was added to the filtrate. **Observation:-** A blue-black solution indicates the presence of tannin.
Test for flavonoid:- Take 5 mL of distilled water and about 0.2 g of plant extract were mixed thoroughly. And 1 mL of 1% AlCl₃ solution was added and shaken. **Observation:-** A light yellow precipitate indicates the presence of flavonoids.
Test for phenol:- About 0.5 g of plant extract was added to 1 mL of 10% FeCl₃ solution. **Observation:-** A deep bluish green colouration was an indication for the presence of phenol.
Test for saponin:- About 0.2 g of plant extract was shaken with 4 mL of distilled water and then heated to boil on a water bath. **Observation:-** Appearance of small bubbles (Frothing) shows the presence of saponin.



Fig.3 Identification test for Turmeric



Fig.4 Identification test for Manjistha



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TEST	Turmeric	Neem	Manjistha	Licorice
Alkaloid	+	+	+	-
Carbohydrates	Nil	-	+	Nil
Flavonoids	+	+	-	+
Phenolics	-	-	+	+
Tannins	-	+	+	+
Saponins	+	+	+	+

2.2 Preformulation Studies

a) Organoleptic Evaluation

Organoleptic evaluation is the assessment of herbal drugs or formulations using the human senses such as sight, smell, taste, and touch. It is a simple and preliminary method used to determine the quality, identity, and purity of raw materials or finished products.

HERBS	COLOUR	TASTE	ODOUR
Licorice	Yellowish-brown	Sweet	Faint
Neem	Dark green	Very bitter	Strong, pungent
Manjistha	Reddish-brown	Bitter & astringent	Slightly aromatic
Turmeric	Bright yellow	Bitter & pungent	Aromatic, characteristic

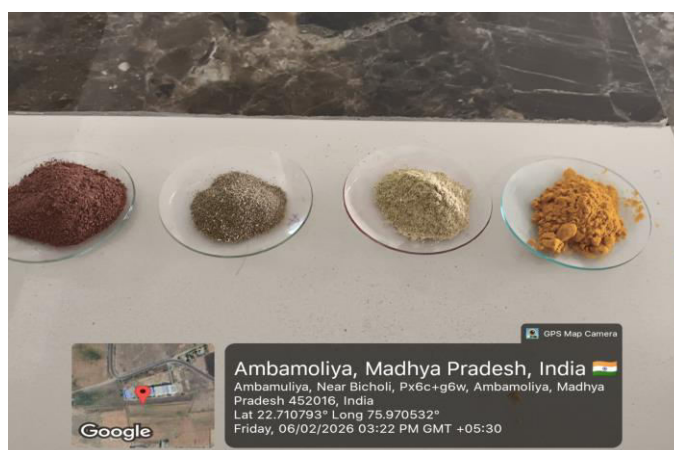


Fig.5 Physical evaluation of herb

s

b) pH- pH is a measure of the acidity or alkalinity of a substance, expressed on a scale from 0 to 14. It indicates the concentration of hydrogen ions present in a solution.

Procedure :- Dip pH paper in 10% herbal aqueous solution and measure with pH scale.



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HERBS	pH
Licorice	4
Neem	5
Manjistha	9
Turmeric	6



Fig.6 pH Determination

c) Moisture Content - Moisture content is the amount of water present in a sample, usually expressed as a percentage of its total weight. It plays an important role in determining the stability and quality of a formulation.

Procedure:- Weigh 3gm herb powder separately in watch glass and put it in hot air oven at 105 degree celsius for 2 hours.

HERBS	WEIGHT BEFORE DRYING	WEIGHT AFTER DRYING
Licorice	3gm	2.81gm
Neem	3gm	2.75gm
Manjistha	3gm	2.73gm
Turmeric	3gm	2.71gm

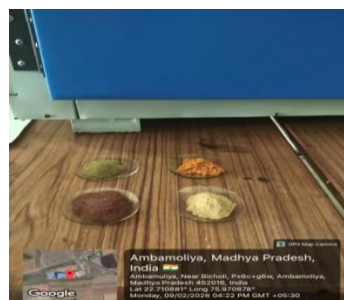


Fig.7 Moisture content Determination



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d) Flow Properties

Bulk Density:- Bulk density is the mass of powder divided by its bulk volume.

Procedure:- Take powdered herb in 10ml measuring cylinder, fill it upto 10ml and then weigh the contents.

Formula:- Bulk Density = Mass of powder/Bulk volume

Tapped Density:- Tapped density is the mass of powder divided by the volume after tapping.

Procedure:- Take powdered herb in 10ml measuring cylinder, fill it upto 10ml and tap it 100 times. Note the volume after tapping.

Formula:- Tapped Density = Mass of powder/tapped volume

Angle of repose:- It is the maximum angle formed between the surface of a pile of powder and the horizontal plane.

Procedure:- Place a glass funnel at 5cm height from bottom platform with the help of burette stand, place a plain paper horizontally, fill the powder sample into the funnel to form a conical heap without disturbance. Measure the height of cone and radius of conical heap.

Formula:- $\theta = \tan^{-1} \left(\frac{h}{r} \right)$

Carr's Index:- Carr's index indicates the compressibility and flow property of powder based on bulk and tapped density.

Formula:- Carr's Index = $\frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$

Hausner's Ratio:- The ratio of tapped density to bulk density, indicating interparticle friction.

Formula:- Hausner's ratio = $\frac{\text{Tapped density}}{\text{Bulk density}}$

III. METHOD OF PREPARATION OF POLYHERBAL CREAM

STEP 1: Preparation of Herbal Extract

- Plant materials are dried, powdered and put aside for maceration extraction with solvents ethanol and water (70:30).
- The extract is filtered and preserved for further step.

STEP 2: Preparation of Oil phase

- Take bees wax, liquid paraffin and stearic acid.
- Heat mixture on water bath at 70°C
- Stir till uniform oil phase is obtained.

STEP 3: Preparation of aqueous phase

- Take distilled water, heat to 70°C.
- Add 1-2 ml Glycerine, mix uniformly.
- Add four extracts (0.5 ml each), stir continuously.

STEP 4: Emulsification

- Maintain temperatures of both the phases.
- Add aqueous phase into oil phase, continuous stirring with glass rod.
- Cream is formed.

STEP 5: Addition of heat sensitive ingredients

- When the mixture cools down to 40°C.
- Add essential oil and preservative.

STEP 6: Evaluation And packaging

- Maintain pH between 5.0-6.5.
- Pack it in a amber colour container and label it properly.



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Fig.8 Emulsification



Fig.9 Cream Formulated

3.1 Composition of Poly Herbal Cream

S. No.	Ingredient	F1	F2	F3
1.	Bees wax	3gm	2.75gm	2.5gm
2.	Liquid paraffin	5 ml	4 ml	4.5 ml
3.	Stearic acid	2gm	1.8gm	1.6gm
4.	Distilled water	10ml	8ml	9ml
5.	Glycerin	2ml	2.5ml	3ml
6.	Methyl paraben	100mg/ Q.S.	200mg/ Q.S.	300mg /Q.S.
7.	Rose oil	1 drop /Q.S.	2 drops /Q.S.	3 drops /Q.S.
8.	Licorice extract	0.5 ml	0.4 ml	0.6 ml

3.2 Evaluation Parameter: -

1) **Physical evaluation:** In this test, the cream was observed for color, odor, texture, state. (3)

2) **Irritancy:** Mark the area (1 cm²) on the left-hand dorsal surface. Then the cream was applied to that area and the time was noted. Then it is checked for irritancy, erythema, and edema if any for an interval up to 24 h and reported. (10,12,14)

3) **Washability:**-A small amount of cream was applied on the hand and it is then washed with tap water. (4)

4) **pH determination:**To prepare 1% w/v concentration, a precisely weighed quantity of cream was distributed in water. To find the pH, a calibrated pH meter was utilized. (2,17)

5) **Phase separation:** Prepared cream was kept in a closed container at a temperature of 25-100 °C away from light. Then phase separation was checked for 24 h for 30 d. Any change in the phase separation was observed/checked.

Observation Table for Evaluation Parameters

S.No.	Parameter	F1	F2	F3
1.	Colour	Creamy Yellow	Creamy Yellow	Creamy Yellow
2.	Odour	Aromatic	Aromatic	Aromatic
3.	Texture	Smooth	Smooth	Smooth
4.	State	Semi-solid	Semi-solid	Semi-solid
5.	Irritancy	Non-irritant	Non-irritant	Non-irritant
6.	Washability	Easily washable	Easily washable	Easily washable
7.	pH	6	6.5	6
8.	Phase separation	No	No	No



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IV. CONCLUSION

The present study successfully formulated and evaluated a polyherbal cream containing Licorice, Neem, Turmeric, and Manjistha for the management of hyperpigmentation. The formulation showed satisfactory physicochemical properties, good stability, non-irritant nature, and skin-friendly pH. The combination of herbal extracts provides synergistic effects, making the cream a safe and effective alternative for improving skin tone and reducing pigmentation

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