

FORMULATION AND EVALUATION OF HERBAL CREAM FOR ANTI AGING ACTIVITY BY USING AMARANTHUS TRICOLOUR LINN

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ABSTRACT

The present research was conducted to formulate and assess a herbal anti-aging cream incorporating the ethanolic leaf extract of *Amaranthus tricolor* Linn. The plant leaves were collected, shade-dried, pulverized into powder, and subjected to Soxhlet extraction using ethanol. The resulting extract was incorporated into a topical cream containing stearic acid, ceto stearyl alcohol, liquid paraffin, triethanolamine, glycerine, methyl paraben, vitamin E oil, rose water, and distilled water. Preliminary phytochemical investigation confirmed the presence of various secondary metabolites, including alkaloids, saponins, phenolic compounds, flavonoids, triterpenoids, tannins, amino acids and proteins, and steroids. The prepared cream was examined for different physicochemical characteristics such as color, odor, homogeneity, wettability, emollience, spreadability, washability, consistency, grittiness, texture, and extrudability. The formulation exhibited desirable physical properties with a smooth and uniform texture, satisfactory consistency, good spreadability, and easy washability. The irritancy study showed the absence of erythema and edema, while the stability assessment indicated acceptable formulation stability. The hydrogen peroxide scavenging assay further confirmed the antioxidant potential of the formulation. Hence, *Amaranthus tricolor* Linn. leaf extract may serve as a promising natural antioxidant for the development of herbal anti-aging topical preparations.

KEY WORDS: *Amaranthus tricolor* linn extract, Anti-aging activity, Soxhlet extraction, Phytochemical screening, Herbal cream.

1.INTRODUCTION

Aging is a complex physiological process characterized by the progressive, degenerative deterioration of the body's primary functions. Hereditary, lifestyle, and environmental factors including xenobiotic pollutants, pathogenic agents, UV irradiation, and dietary toxins all these causes the unavoidable progression. Senescence and the aging process are associated with various internal and external signs and symptoms, including wrinkles, dry skin, diabetes mellitus, advanced atherosclerosis, oncogenesis, and neurodegenerative diseases. A principal mechanism driving this age-related cellular damage is the induction of oxidative stress. This state arises from a critical homeostatic imbalance between pro-oxidants and antioxidants, producing highly volatile metabolic byproducts, specifically reactive oxygen species and reactive nitrogen species ⁽¹⁾.

1.2. CREAM

Creams are topical medications that are administered topically to the skin. Creams are described as "viscous liquid or semi-solid emulsions of either the oil-in-water or water-in-oil type" dosage forms with varying water and oil consistency. Creams are used for medicinal or cosmetic objectives, such as cleansing, beautifying, enhancing looks, and providing protection. These topical formulations are used to administer the medication to the mucous membrane or the skin underlying layer in a localized manner. Ayurvedic, herbal, or allopathic creams are used by people to treat various skin diseases. Based on their phases, creams can be categorized as either o/w or without types of emulsion. Traditionally, the term "cream" has been used to describe semisolid formulations that are either oil-in-water (such as vanishing cream) or water-in-oil (such as cold cream) ⁽²⁾.

ADVANTAGES:

1. It is the easiest method for giving medication.
2. It does not cause harm to other organs.
3. It is easy and convenient to use.
4. It can help slow down the signs of aging.

DISADVANTAGES:

1. Itchiness of the skin.
2. Certain medications have poor skin penetration.
3. Allergy responses are possible.
4. Compared to other semisolid preparations, they are less hydrophobic, which increases the danger of contamination ⁽³⁾.

1.3. AMARANTHUS TRICOLOR LINN

Amaranthus tricolor Linn originally originates from tropical Asia. It is the most important species of *Amaranthus* in south and south-east Asia and is considered one of the primary leafy vegetables in these regions. The leaves of red amaranth are rich in minerals like iron, calcium, magnesium, potassium, and zinc, as well as proteins, dietary fibre, fat, and carbohydrates. These leaves also contain other nutrients such as vitamin C, betalains, betaxanthins, and betacyanin's. Additionally, red amaranth leaves contain various phytochemicals like alkaloids, glycosides, phenolic acids, flavonoids, amaranthine, and tannins. These compounds have several health benefits, including helping to control blood sugar levels, fight free radicals, reduce bacterial growth, lower inflammation, and help manage depression ⁽⁴⁾.

2. PLANT PROFILE

Amaranthus tricolor Linn is a notable purple-red leafy vegetable that is widely eaten across much of India, especially in the states of Bihar, Jharkhand, and West Bengal. It is also present in other tropical countries, including South Africa. The leaves are rich in proteins and essential minerals such as iron, calcium, zinc, as well as vitamins A and C ⁽⁵⁾.

2.1. SYNONYMS:

2.1.1. MAJOR SYNONYMS:

- ❖ *Amaranthus gangeticus* Linn
- ❖ *Amaranthus melancholicus* Linn
- ❖ *Amaranthus polygamus* Linn
- ❖ *Amaranthus tristis* Linn⁽⁶⁾

2.2. TAXONOMICAL CLASSIFICATION:

- ❖ Domain: Eukaryota
- ❖ Kingdom: Plantae
- ❖ Phylum: Tracheophytes
- ❖ Order: Chenopodiaceae
- ❖ Family: Amaranthaceae
- ❖ Subfamily: Amaranthoideae
- ❖ Genus: *Amaranthus*
- ❖ Species: *Amaranthus tricolor*

2.3. MORPHOLOGICAL DESCRIPTION:

2.3.1. ROOTS:

- **Shape:** Cylindrical and fibrous fracture
- **Length:** 10-12 cm and 0.5-1 cm thickness
- **Colour:** Yellow colour with rootlets



Fig. 1 Red spinach Root

2.3.2. STEM:

- **Colour:** Dark purple
- **Shape:** Cylindrical with longitudinal ridges and furrows.
- **Length:** 20-30 cm height and 0.3-1.5 cm wide

2.3.3. LEAVES:

- **Colour:** Purplish pink
- **Shape:** Rhomboid, ovate, lanceolate, elliptical, petiolate, membranous.
- **Length:** 5-12 cm long and 2.5-7cm wide
- **Taste:** Mild, earthy, and nutty flavour



Fig. 2 Red spinach leaves

2.3.4. FLOWERS:

- **Shape:** Lanceolate
- **Colour:** Pinkish
- **Odour:** Scentless



Fig. 3 Red spinach flowers

2.3.5. SEED:

- **Shape:** Tiny, Round, biconvex
- **Colour:** Shiny black or dark brown
- **Length:** 1-1.5 mm in diameter ⁽⁷⁾



Fig. 4 Red spinach seeds

3. MATERIALS AND METHODS

3.1. COLLECTION:

From June to July, *Amaranthus tricolor* Linn leaves were gathered from the regions of Hosur in Tamil Nadu, India. After collecting, the leaves were cleaned and separated from the stem and roots for processing.

3.2. DRYING:

The drying process involves shade drying of the leaves at room temperature in the lab until complete removal of moisture content, which takes around 7 days. After drying, the leaves were pulverized into fine powder and stored in an airtight container ⁽⁸⁾.

3.3. SOXHLET EXTRACTION:

It is one of the methods used to extract the active ingredients from the plant. When the solvent in the flask is heated and its vapours rise and condense, this method entails placing the powdered crude drug onto a filter-paper thimble and repeatedly washing it through the condensed liquid.

PROCEDURE:

After they were pounded into a fine powder after drying. Then, using ethanol as the solvent, the powdered leaves were extracted using Soxhlet. Thirty grams of powdered leaves were placed in the Soxhlet thimble for the extraction, and three hundred ml of ethanol were added to the round-bottom flask. The extraction process was set up with a condenser and operated for 6 to 8 hours. The ethanol extract was collected for later use after completion ⁽⁹⁾.



Fig. 5 Soxhlet extraction

4. MATERIALS USED IN CREAM FORMULATION:

- Active ingredient
- Stearic acid
- Ceto stearyl alcohol
- Triethanolamine
- Methyl paraben
- Glycerine
- Vitamin E oil
- Rose water
- Distilled water

4.1. ACTIVE INGREDIENT:

- Colour: Greenish-red
- Appearance: Liquid or semi-solid extract
- Odour: Characteristic herbal odour ⁽¹⁰⁾



Fig. 6 Leaf powder

4.2. STEARIC ACID:

- Chemical formula: C₁₈H₃₆O₂
- Colour: White or off-white
- Appearance: Crystalline solid or white powder
- Odour: Pungent and fat like odour
- Melting point: 69.3 °C (156.7 °F)
- Boiling Point: 361 °C (681.8 °F) ⁽¹¹⁾



Fig. 7 Stearic Acid

4.3. CETO STEARYL ALCOHOL:

- Colour: White to off-white solid
- Appearance: Usually sold as pellets, flakes, or pastilles.
- Odour: Mild, faint characteristic soapy or fatty odour.
- Melting Point: 48 °C to 56 °C (118 °F to 133 °F).
- Boiling Point: > 300 °C (> 572 °F) ⁽¹²⁾.



Fig. 8 Ceto stearyl alcohol

4.4. TRIETHANOLAMINE:

- Chemical formula: C₆H₁₅NO₃
- Colour: Colourless to pale yellow
- Appearance: Viscous, clear to light yellow liquid
- Odour: Faint, mild ammonia-like odour
- Melting Point: 20.5 °C (68.9 °F)
- Boiling Point: 335.4 °C (635.7 °F)

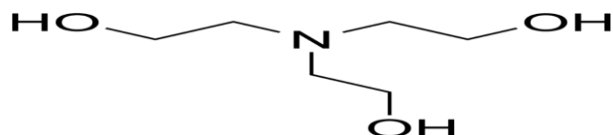


Fig.9 Triethanolamine ⁽¹³⁾

4.5. METHYL PARABEN:

- Chemical formula: C₈H₈O₃
- Colour: White or colourless
- Appearance: Crystalline powder or small crystals
- Odour: Odourless
- Melting Point: 125°C to 128°C

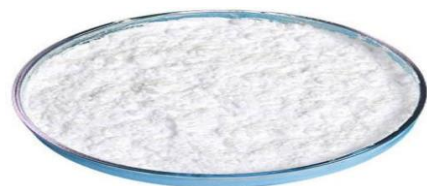


Fig. 10 Methyl paraben ⁽¹⁴⁾

4.6. GLYCERINE:

- Chemical formula: C₃H₈O₃
- Colour: Clear and colourless
- Appearance: Syrupy with a thick and viscous consistency
- Odour: Odourless

- Melting point: 18°C
- Boiling point: 290°C

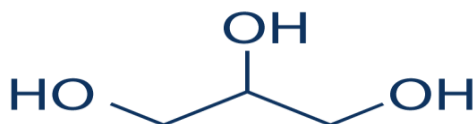


Fig. 11 Glycerine ⁽¹⁵⁾.

4.7. VITAMIN E OIL:

- Chemical formula: C₂₉H₅₀O₂
- Colour: Pale yellow to a warm golden-amber colour
- Odour: Mild earthy or nutty aroma
- Appearance: It is extremely thick, viscous and syrupy in its pure form
- Melting point: Approximately 2–3°C
- Boiling point: Decomposes at high temperature

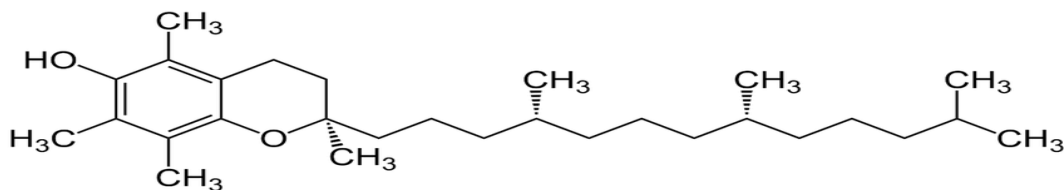


Fig.12 Vitamin E oil

4.8. ETHANOL:

- Colour: Colourless
- Appearance: Clear, volatile, mobile liquid
- Odour: Characteristic alcoholic odour
- Melting Point: 114.1 °C
- Boiling Point: 78.37 °C

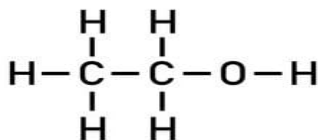


Fig. 13 Ethanol

5. CREAM PREPARATION:

Preparation of oil phase: Ceto stearyl alcohol, liquid paraffin, and stearic acid were weighed and heated together in a beaker using a hot plate.

Preparation of Aqueous phase: Glycerine, triethanolamine, and methyl paraben were dissolved in water and melted in a different beaker.

Emulsification Process: Rose water, vitamin E oil, and the heated aqueous phase were gradually added to the oil phase while being continuously stirred either by hand or with a mechanical stirrer until a homogenous emulsion was created. The end product is packed and labelled ⁽²³⁾.



Fig.14 Cream

5.1. FORMULATION TABLE:

S.NO	INGREDIENTS	QUANTITY NEEDED FOR (50g)	USES
1.	<i>Amaranthus tricolor</i> linn extract	2.5g	Antioxidant activity
2.	Stearic acid	6g	Cream base
3.	Ceto stearyl alcohol	1.5g	Consistency
4.	Liquid paraffin	4.7ml	Skin softener
5.	Triethanolamine	0.7ml	Emulsifier
6.	Methyl paraben	0.10g	Preservative
7.	Glycerine	2.0ml	Humectant
8.	Vitamin E oil	0.27ml	Antioxidant activity
9.	Rose water	Few drops	Flavouring agent
10.	Water	QS as needed	Vehicle

Table.1 Formulation of Herbal cream

6. ANALYSIS OF AMARANTHUS TRICOLOR LINN EXTRACT

TEST FOR ALKALOIDS			
S.NO	EXPERIMENT	OBSERVATON	INFERENCE
1.	Dragendroff test: Add sample in aqueous HCL (5mL), then filtered, and reacted with Dragendroff's solution.	A red precipitate is formed	Presence of alkaloids
TEST FOR SAPONIN			
1.	4 mL of distilled water is added to each 2 mL of extract sample.	After shaking vigorously foam is produced	Presence of saponin
TEST FOR PHENOLS			
1.	To 2 mL of the extract, add 2 mL of a 5% ferric chloride (FeCl ₃) solution.	A blue colour is formed	Presence of phenol
TEST FOR FLAVONOID			
1.	Add a few drops of 20% NaOH to 2 mL of the extract.	Formation of yellow colour	Presence of flavonoid
TEST FOR TRITERPENOID			
1.	Combine 0.5 mL of acetic anhydride and chloroform with 2 mL of the extract. Add a few drops of concentrated sulfuric acid (H ₂ SO ₄) with caution.	A reddish-brown precipitate is produced	Presence of terpenoids
TEST FOR TANNINS			
1.	Add 2mL of 10% alcoholic ferric chloride solution to 2mL of filtered extract.	Development of a blackish blue colour	Presence of tannins.
TEST FOR AMINO ACID			
1.	Add 1ml of extract with few drops of aqueous Ninhydrin solution and keep in a boiling water bath for 2 mins.	Formation of purple colour	Presence of amino acid.

TEST FOR STERIOD			
1.	2mL of chloroform were added to each 2mL of extract sample, followed by 2mL of concentrated sulfuric acid.	The chloroform layer turned to red while the acid layer turned to greenish yellow colour	Presence of steroids.

Table.2 Qualitative Analysis of Phytochemical constituents ^(16,17)



Fig.15 Phytochemical screening

7. EVALUATION TEST FOR HERBAL CREAM

1. PHYSICOCHEMICAL EVALUATION:

To assess the qualities of the formulated cream by observing the characteristics like texture, uniformity, colour, odour, and physical appearance.

PROCEDURE:

- ❖ Place one to two grams of cream on a clean white glass dish.
- ❖ Visually check for colour, phase separation, and the existence of any particles.
- ❖ Look for any pleasant, subtle, or strong smell in the sample.
- ❖ Check the texture of your skin by touching it to feel its smoothness, grittiness, and consistent spreadability.

2. PH MEASUREMENT:

A calibrated digital pH meter is used to measure the cream's acidity or alkalinity in order to verify skin compatibility and stability. The permissible skin range of 5.0–7.0 is ensured to avoid skin irritation upon application.

PROCEDURE:

- ❖ Calibrate the PH meter with standard buffers (PH 4,7,9) at room temperature.
- ❖ Weigh 1g of cream and disperse in 10ml distilled water and stir for 10min.
- ❖ Immerse electrode and record PH after stabilization (30-60s). Record the pH value.

3. SPREADABILITY:

It is carried out to evaluate the spreading of cream on skin under a small pressure. It is calculated by using the formula, $S=(M \times L)/T$

Where;

- S- Spreadability
- M-Mass tied to upper slide (g)
- L-Length moved (cm)
- T-Time (s)

PROCEDURE:

- ❖ Between two glass slides, place a fixed amount of cream.
- ❖ After applying a known weight, measure the time it takes for the upper slide to move a specific distance.

4. WASHABILITY:

It is carried out to evaluate how easily the cream can be washed off with water and non-greasiness.

PROCEDURE:

- ❖ Apply a small amount of cream to the skin.
- ❖ Allow to set for 10 minutes.
- ❖ Wash with water and observe the ease of removal.

5. AFTER FILL TEST:

For observing any changes in cream's characteristics after filled into the containers.

It ensures the container durability and long-term product stability.

PROCEDURE:

- ❖ Examine the filled packaging for 24 to 48 hours.
- ❖ Check for leakage or phase separation.
- ❖ Look for any changes in product texture.

6. IRRITANCY TEST:

To check any irritant or allergic reaction of cream when applied to the skin.

This test ensures the safety of the cream for human use.

PROCEDURE:

- ❖ Apply a small amount of cream on a patch of skin and leave for 24 hours.
- ❖ Observe any allergic reactions like redness, swelling, itching, or rash.

7. GRITTIENESS:

It is used to identify if the cream contains any coarse particles, and ensures the smooth texture for easy application on the skin.

PROCEDURE:

- ❖ Apply a small amount of cream on your fingertip between fingers and rub gently.
- ❖ Check the presence of gritty or coarse particles and visually check under the microscope to confirm the presence of particles.
- ❖ Record the results as smooth, slight grittiness, and high gritty.

8. STABILITY TEST:

To assess the formulation's long-term physical and chemical stability which establishes the product's shelf life and storage needs.

PROCEDURE:

- ❖ Cream samples should be stored in various settings.
- ❖ Standard temperature
- ❖ Keeping cold
- ❖ Higher. temperature ($40^{\circ}\text{C} \pm 2^{\circ}\text{C}$)
- ❖ For one to three months, observe.
- ❖ Examine whether the colour, odour, pH, consistency, and phase separation have changed.

9. CONSISTENCY:

It is carried out to assess the thickness, uniformity and firmness of the cream and helps in stability measures which ensures the proper drug delivery to the skin.

PROCEDURE:

- ❖ A fixed amount of cream was taken on a slide.
- ❖ Press gently with another slide and observe the spreading of the cream.
- ❖ Record whether the cream is too thick, to thin and ideal ⁽¹⁸⁾.

10. HYDROGEN PEROXIDE ASSAY:

The human body can absorb hydrogen peroxide thru touch with the skin or eyes, as well as by breathing in vapour or mist. The quick breakdown of H_2O_2 into oxygen and water can result in hydroxyl radicals (OH), which can start peroxidation and harm the body's DNA.

PROCEDURE:

1. Preparation of Reagents and solutions:

- **Phosphate buffer (pH 7.4, 50 mM):**

In distilled water, dissolve potassium dihydrogen phosphate (KH_2HPO_4) and disodium hydrogen phosphate (Na_2HPO_4). Use a calibrated pH meter to get the pH down to 7.4.

• **Solution of hydrogen peroxide (40 mM):**

In 100 mL of the prepared 50 mM phosphate buffer (pH 7.4), pipette 0.4 mL of commercial hydrogen peroxide (30% w/v) and dissolve it. Before using, prepare fresh.

2. Concentration Gradient & Assay Mixture setup:

A series of direct dilutions was performed in order to keep the total reaction volume of 10 mL per tube constant. Various amounts of the primary extract stock (2, 4, 6, 8, and 10 mL), a constant volume of 40 mM H₂O₂ (0.6 mL), and 50 mM phosphate buffer (pH 7.4) were combined to create test reaction mixtures.

Tube 1: 2 mL extract + 0.6 mL H₂O₂ + 7.4 mL buffer

Tube 2: 4 mL extract + 0.6 mL H₂O₂ + 5.4 mL buffer

Tube 3: 6 mL extract + 0.6 mL H₂O₂ + 3.4 mL buffer

Tube 4: 8 mL extract + 0.6 mL H₂O₂ + 1.4 mL buffer

Tube 5: 10 mL extract + 0.6 mL H₂O₂ + 0.0 mL buffer

0.6 mL of 40 mM H₂O₂ and 9.4 mL of phosphate buffer without extract were used to create a control solution.

The UV-Vis spectrophotometer was calibrated using a blank solution that contained only phosphate buffer without hydrogen peroxide.

The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\text{Percentage scavenged (H}_2\text{O}_2) = \left[\frac{(A_i - A_t)}{A_i} \right] \times 100$$

Where,

A_i is the absorbance of control

A_t is the absorbance of test

S.NO	Concentration (µg/mL)	Absorbance at 230nm	Percentage (%) Inhibition
1.	Control (A0)	0.500	-
2.	2	0.495	1.00%
3.	4	0.417	16.60%
4.	6	0.398	20.40%
5.	8	0.282	43.60%
6.	10	0.199	60.20%

Table.3 Hydrogen peroxide scavenging activity of sample vs concentration ⁽¹⁹⁾

8. RESULTS AND DISCUSSION

FORMULATION:

The formulation of herbal cream involves in the use of leaves extract of *Amaranthus tricolor linn* as an active ingredient. The process involves preparation of oil phase and aqueous phase and combining them to create the cream base, and then adding the active ingredient. The finished product is transferred into a suitable container.

EVALUATION:

To verify the quality, safety, and effectiveness, the physicochemical qualities were evaluated for qualitative parameters such as organoleptic properties, pH, viscosity, spreadability, skin irritation test, and hydrogen peroxide assay.

8.2. QUALITATIVE ANALYSIS OF AMARANTHUS TRICOLOR LINN EXTRACT:

S.NO	SECONDARY METABOLITES	RESULTS (PRESENCE/ ABSENCE)
1.	Alkaloids	+
2.	Saponin	+
3.	Phenols	+
4.	Flavonoids	+
5.	Triterpenoids	+
6.	Tannins	+
7.	Amino acids & proteins	+

8.	Steroids	+
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Table.4 Qualitative Analysis of Phytochemical Constituents

8.4. TEST FOR IRRITANCY:

PARAMETER	ERYTHEMA	IRRITANT	EDEMA
Test for irritant	None	None	None

Table.5 Irritancy Test

8.5. STABILITY EVALUATION:

S.NO	PARAMETER	FORMULATION RESULT
1.	Stability test	pass

Table.6 Stability Test

8.6. HYDROGEN PEROXIDE ASSAY:

S.NO	PARAMETER	FORMULATION RESULT
1.	Anti-oxidant	pass

Table.7 Anti-oxidant Parameters

9. CONCLUSION

The present study successfully developed a herbal anti-aging cream containing ethanolic leaf extract of *Amaranthus tricolor* Linn. The preliminary phytochemical screening confirmed the presence of various bioactive constituents such as alkaloids, saponins, phenols, flavonoids, triterpenoids, tannins, amino acids and proteins, and steroids. The formulated cream exhibited satisfactory physicochemical properties, including smooth texture, good homogeneity, uniform spreadability, easy washability, appropriate consistency, and absence of grittiness. The formulation also showed no signs of erythema or edema during the irritancy evaluation and passed the stability assessment. Furthermore, the hydrogen peroxide assay indicated antioxidant activity of the formulation. These findings suggest that *Amaranthus tricolor* Linn. leaf extract has potential as a natural antioxidant ingredient in topical anti-aging preparations. Thus, the developed herbal cream may serve as a promising plant-based approach for supporting skin care and reducing oxidative-stress-related effects associated with skin aging.

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