

Phytochemical Screening and Anti-Ageing Potential of Herbal Extracts in A Topical Cream Formulation

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ABSTRACT

Background: Calendula, chamomile, and butterfly pea contain a high concentration of antioxidants that rejuvenate skin and provide relief from irritation. Based on this information, we can assume that extracts of these plants can be used to make a sustainable anti-ageing cream as a natural alternative to existing skincare products. **Objective:** This research aims to formulate a topical cream using extracts of calendula, chamomile, and butterfly pea. The work also includes tests of the phytochemical profile and evaluation parameters of the cream. **Materials and Methods:** In this study, we assessed the content of flavonoids, phenolics, tannins, saponins, and alkaloids by performing qualitative phytochemical screening on methanolic extracts of *Clitoria ternatea*, *Calendula officinalis*, and *Matricaria chamomilla*. Using standard methods, we emulsified the mixed herbal extracts to produce an oil-in-water cream. Once prepared, the cream formulation was evaluated for organoleptic properties, pH, viscosity, homogeneity, spreadability, and stability under a variety of storage conditions. **Results:** All three herbal extracts were shown to have significant quantities of phenolic compounds and flavonoids upon phytochemical analysis. Additionally, the formulated cream proved to have desirable organoleptic qualities that include a pH of 4.5- 5.2, stable viscosity, and good spreadability. It also proved to remain homogeneous with no evidence of phase separation or microbial growth over the duration of the 30-day stability study.

Key Words:

Butterfly pea (*Clitoria ternatea*), Calendula (*Calendula officinalis*), Chamomile (*Matricaria chamomilla*), Phytochemical screening, Herbal Anti-aging cream

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1. INTRODUCTION

The two principal layers of the skin are the epidermis and dermis, which together comprise its mass. The outermost layer of the epidermis, known as the stratum corneum, is a selectively permeable barrier that effectively shields the body from exposure to chemical and physical factors, including sunlight, dust, and air pollution. For the epidermis to remain an effective protective layer, the pH level must remain in equilibrium. Skin becomes excessively sensitive, irritated, or dry when its pH level is either too high or too low. Accordingly, maintaining a pH level between 4.5 and 8.0 is vital. Uncontrolled free radicals have a destructive effect on

biological tissue and can lead to certain symptoms, including redness, pigmentation, and an increased risk of cancer. The decrease of antioxidative enzymes in our bodies, such as glutathione peroxidases, catalyze, superoxide dismutase (SOD), and glutathione S-transferase, is responsible for the observed impact¹. Fig. 1 depicts the aging process.

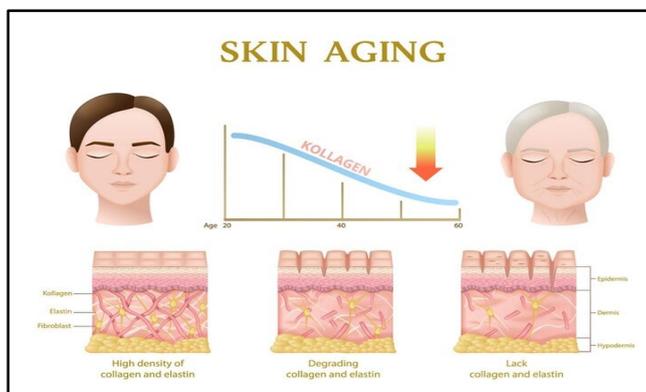


Fig. 1: Process of aging

The human body experiences an unavoidable decline in tissue structure, function, and other components as its ages. This process of aging, which is irreversible, eventually results in death. However, there are steps that can be taken to slow this decline. Through slowing aging, it may be possible to prolong human lifespans and maintain youthfulness. Skin aging is a complex process that can be understood from a clinical perspective. Clinical evidence shows that there are two types of skin aging, “photo-aging” and “sequential aging.” Each type presents unique clinical characteristics, and both forms of skin aging cause damage to proteins and DNA at the cellular level. With sequential skin aging, changes in physiology, such as reduced lipid synthesis, cause skin to become dry and wrinkled. Photoaging, however, is the result of exposure to UV light, and it causes skin disorders².

1.1 Anti-aging: Aging involves complex biological processes that are influenced by both genetic and environmental factors. Physiologically active substances and micronutrients may have anti-aging properties by concentrating on intracellular pathways. Understanding the molecular origins of aging makes it easier to develop effective anti-aging treatments, lower the incidence of age-related diseases, and increase longevity³. In ancient times, natural remedies were the primary means of treating aged skin. Traditional ways to maintain youthful-looking skin sometimes involved the use of herbs, oils, and other natural ingredients⁴. Calendula, chamomile, and butterfly pea flowers are traditional skin care components due to their anti-inflammatory, antibacterial, and antioxidant properties. The purpose of this study is to create an herbal anti-aging cream using these three botanical extracts and assess its stability and effectiveness⁴. *Clitoria ternatea*, *Calendula officinalis*, and *Matricaria chamomilla*, respectively, are rich in anthocyanins, triterpenoids and carotenoids, and apigenin and azulene.

2. PLANT PROFILE

1. Butterfly pea flower (*Clitoria ternatea* L.): The picture of Butterfly pea flower is shown in Fig. 2.



Fig. 2: Butterfly pea flower

Biological sources: The biological source of the butterfly pea flower is the *Clitoria ternatea* plant.

Family- Fabaceae

Pharmacological activities:

- Stress reliever
- As anti-inflammatory
- Anti-aging
- Anti-oxidant
- Keeps skin glowing and healthy

Useful part: Flowers ⁴

Chemical constituents: Butterfly pea flowers contain many types of phytochemicals which are responsible for their antioxidative and protective qualities. Anthocyanins, such as ternatins which give the flowers their color, are especially important antioxidants. These flowers also contain alkaloids, saponins, triterpenoids, and flavonoids such as myricetin, kaempferol, and quercetin ⁵.

2. Calendula (*Calendula officinalis* L.): The picture of Calendula flower is shown in Fig. 3.



Fig. 3: Calendula flower

Biological sources: The biological source of the calendula flower is the *Calendula officinalis* Linn plant.

Family- Asteraceae

Pharmacological activities:

- Heals cuts and dry skin.
- Excellent for dry and damaged skin.
- Reduces acne and rashes.

Useful part: Flower ⁶

Chemical constituents: Multiple constituents, including flavonoid, triterpenoid, saponin, carotenoid, volatile oil, steroid, amino acid, and quinine, in a particular item exhibit skincare-enhancing property. Their presence contributes to multiple beneficial biological effects. For instance, it is well established that some plants contain constituents that are responsible for the anti-inflammatory, anti-cancer, anti-diabetic, wound-healing, and antioxidant activity of the plant ⁷.

3. Chamomile (*Matricaria chamomilla* L.): The picture of Chamomile flower is shown in Fig. 4.



Fig. 4: Chamomile flower

Biological source: The biological source of the chamomile flower is the *Matricaria chamomilla* Linn. plant.

Family- Asteraceae

Pharmacological activities:

- For calming and relaxing
- Anti-inflammatory and Anti-oxidant
- Soothing and skin irritation
- Gastro-protective effect
- Antidiabetic activity

Useful part: Flower ⁸

Chemical constituents: The study shows that includes terpenes, organic acids, polysaccharides, coumarins, flavonoids, and volatile oil. It has antioxidant, anti-inflammatory, anti-hypertensive, anti-depressant, anti-cancer, and anti-infective properties ⁹.

3. MATERIALS AND METHODS

3.1. Collection of plant materials: The plant material BPF, calendula, chamomile flowers were collected from Geeta nursery, Raipur (C.G.).

3.2. Chemicals and reagents

Emulsifying wax, cetosteryl alcohol, stearic acid, shea butter, ascorbic acid, glycerine, paraben, distilled water, perfume was acquired at the local market.

3.3. Ultrasound-Assisted Extraction of flowers (UAE): Ultrasound-assisted extraction (UAE) has been revealed to be an effective method for extraction due to the shorter extraction time, lower solvent usage and reduced cost. The universal solvent was typically selected due to its high polarity. This high polarity also allowed it to be a universal solvent that could extract many different polar compounds. The flowers of calendula, chamomile, and butterfly pea were dried at room temperature and then pulverized. The mixture was then subjected to sonicate for 90 min at 37 °C. After this step, 15 g of BPF, calendula, and chamomile were added to 250 ml of distilled water, and the mixture was filtered with filter paper. The solvent was evaporated under vacuum. Following the removal of the solvent, the resulting filtrate was weighed. Phytochemical tests were performed on the extracts ^{1,10-12}. Fig. 5 shows the UAE method for BPF, calendula, and chamomile flowers.



Fig. 5: UAE of BPF, calendula and chamomile flower

3.4. Preliminary Screening of Extracts

1. Test for Alkaloids:

- **Dragendorff's test:** Approximately 1 ml of extract and a few drops of Dragendorff's test reagent yield a precipitate that is a reddish-orange color, indicating the presence of alkaloids.
- **Mayer's test:** 1 ml of filtrate was mixed with a few drops of Mayer's reagent; the yellowish ppt indicates the presence of alkaloids.

2. Test for Flavonoids:

- **FeCl₃ test:** A few drops of FeCl₃ solution added to the extract resulted in a vivid green and black precipitate that indicated the presence of flavonoids.
- **Lead acetate test:** When a few drops of lead acetate solution were added to the filtrate, the presence of flavonoids was indicated by a yellowish ppt.
- **Alkaline reagent test:** A few drops of NaOH solution were added to 1 ml of filtrate, formation of bright yellow and disappeared when the diluted HCl acid was added. This indicates the presence of flavonoids.

3. Test for Terpenoids: In order to create a layer, plant extract was combined with concentrated H₂SO₄ and 2 ml of chloroform. This reaction shows that the presence of terpenoids is the result of the formation of a reddish-brown coloration at the contact area.

4. Test for Tannins and Phenols: The test extract was taken in water, warmed and filtered. 5 ml of the filtrate were allowed to react with 1 ml of 5% ferric chloride solution. Dark green or deep blue color shows the presence of tannins and phenols.

5. Test for the Steroids: The extract was treated with 0.5 ml of chloroform, followed by a gradual addition of concentrated sulfuric acid. The steroid content of the extract is evidenced by the appearance of a green-bluish color.

6. Test for Saponin: The saponins in the crude extract were identified by combining it with distilled water in a test tube, shaking the solution vigorously, and verifying the formation of a stable foam.

7. Test for Glycosides:

- **Killer- killani test:** Place a sample in a flask. Mix with CHCl_3 and put the sample in a flask. Set it to high heat and allow it to evaporate. Add 0.4 ml of glacial acetic acid, along with a trace amount of FeCl_3 . Carefully add H_2SO_4 . If a brown precipitate is formed, this indicates the presence of glycosides.

8. Test for Anthocyanin: When 2 ml of filtrate is treated with 2 ml of 2N HCl and 1 ml of NH_3 , the formation of a pinkish red color indicates the presence of anthocyanin.

9. Test for Quinone: A crimson color indicates that Quinine is present when 1 ml of concentrated H_2SO_4 reacts with 1 ml of a filtrate.

10. Test for reducing sugar:

- **Benedict's test:** Benedict's reagent was applied to the filtrate. Boil for five minutes in a thermostatic water bath. Formation of brick-red ppt indicates the presence of reducing sugars^{13,14}.

3.5. Formulation of Anti-aging Cream: An oil-in-water (o/w) emulsion was prepared using:

Oil phase: Stearic acid, Emulsifying wax, Ceto steryl alcohol, Shea butter

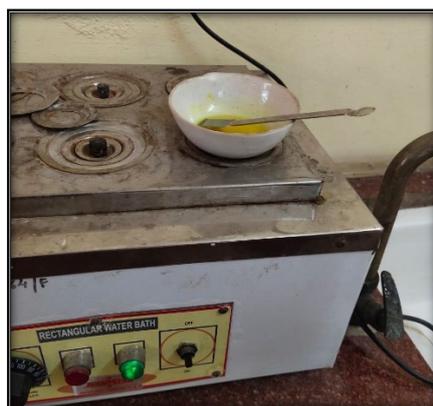
Aqueous phase: Glycerine, Flower extracts, Distilled water, Ascorbic acid

Cooling phase: Methyl paraben, perfume

3.5.1. Preparation method: The herbal anti-aging cream was prepared by first heating the oil phase to 70°C , followed by heating the aqueous phase to 75°C . The aqueous phase was then slowly added to the oil phase under continuous stirring until the homogeneous cream mixture formed. The therapeutically active ingredient, methylparaben, a preservative, was added after cooling to 40°C . Perfume was added just before the finished product was transferred to a suitable container. Preparation and evaluation of the herbal anti-aging cream⁴ was completed. Formulation of the cream was prepared and shown in Fig. 6 and the list of ingredients used for preparation of the formulation was shown in Table 1.

Table 1: List of ingredients used for formulation

| S. No. | Ingredients | Properties | Cream |
|--------|--------------------------|-------------------|--------|
| 01. | BPF Extract | Anti-aging | 0.3 ml |
| 02. | Calendula flower extract | Anti-inflammatory | 0.2 ml |
| 03. | Chamomile flower extract | Soothing | 0.2 ml |
| 04. | Glycerine | Humectants | 0.3 gm |
| 05. | Shea butter | Moisturizer | 0.3 gm |
| 06. | Emulsifying wax | O/W Base | 0.4 gm |
| 07. | Ceto steryl alcohol | Thickener | 0.4 gm |
| 08. | Stearic acid | Thickener | 0.2 gm |
| 09. | Ascorbic Acid | Sun protection | 0.5 gm |
| 10. | Methyl paraben | Preservative | 0.5 gm |
| 11. | Dis. Water (q.s.) | Anti-oxidant | 6 ml |

**Fig. 6: Formulation of Herbal anti-aging cream**

3.6. Evaluation Test for Herbal Anti-Aging Cream

1. Visual Evaluation

The physical characteristics of the produced formulations, such as appearance, color, fragrance, and consistency, were evaluated.

2. Homogeneity

The homogeneity of the cream formulation was determined by visual assessment.

3. pH

The pH meter was calibrated with a standard buffer solution. Using a digital pH meter, we measured the pH of a 0.5 g cream sample that had been dissolved in 50 ml of distilled water.

4. Viscosity

The viscosity of the cream was measured using a Brookfield viscometer. Ten grams of cream was placed in a beaker, and a spindle was dipped into the cream for about five minutes. Then, a reading was taken ¹⁵.

5. Spreadability

In order to determine the spreadability, the amount of sample was sandwiched between two slides and squeezed to a consistent thickness by applying a certain weight for a certain amount of time. The spreadability was the amount of time required for the two slides to separate. The better the spreadability, the less time it took for the slides to separate. The following formula was used to determine spreadability:

$$S=M \times L / T$$

Where,

S = Spreadability

M = Tide weight on upper slide

L = Length of glass slide

T = Time taken for the separation of slides

6. Washability

The mixture was applied to the skin, then the ease of washing it off with water was assessed ^{16,17}.

7. Non-irritancy

A formulation of herbal anti-aging cream was tested on human skin, and the results were noted.

8. Removal

The applied area was washed with tap water to test how easy it was to remove the cream.

9. Stability Study

The stability of an herbal anti-aging cream was measured for four weeks at three temperatures: 25°C, 37°C, and 44°C ¹⁸⁻²⁰.

4. RESULTS AND DISCUSSIONS

4.1 Preliminary screening

Preliminary phytochemical analyses revealed various bioactive constituents that promote skin health. We used extracts from butterfly pea, calendula, and chamomile flowers to screen for the presence of certain phytochemicals. The results of this testing are summarized in Table 2. The extracts we tested, butterfly pea flower (BPF), calendula, and chamomile all displayed high levels of alkaloids, flavonoids, phenols, and tannins. These are generally associated with

antibacterial, anti-inflammatory, and antioxidant properties. While BPF, and calendula shared similar phytochemical diversity, each also contained some unique constituents. For example, only BPF contained anthocyanins, which provide excellent antioxidant properties and make it well-suited for anti-aging products. Meanwhile, saponin, glycosides, steroids, and quinine were found in both calendula and BPF, but not in chamomile. This indicates that chamomile possesses the lowest phytochemical diversity among the three plants. Lastly, chamomile is the only extract that contained reducing sugars, which suggests that it may also contribute to skin hydration.

Table 2: Results of the preliminary test of herbal extracts

| S. No. | Preliminary test | BPF Extract | Calendula flower extract | Chamomile flower extract |
|--------|------------------|-------------|--------------------------|--------------------------|
| 01. | Alkaloidal | + | + | + |
| 02. | Flavonoids | + | + | + |
| 03. | Phenol | + | + | + |
| 04. | Saponin | + | + | - |
| 05. | Tannins | + | + | + |
| 06. | Glycosides | + | + | - |
| 07. | Reducing sugar | - | - | + |
| 08. | Steroids | + | + | - |
| 09. | Quinine | + | + | - |
| 10. | Anthocyanin | + | - | - |

4.2 Evaluation

Based on factors such as appearance, pH, spreadability, washability, and irritation, it can be concluded that the laboratory-formulated herbal anti-aging cream meets all necessary characteristics. Therefore, the resulting formulation might be successfully employed as a cosmetic formulation for creating radiant and healthy skin. In addition, the organoleptic evaluation of the herbal anti-aging cream is summarized in Table 3, while the viscosity, pH, and spreadability test results are given in Table 4.

Table 3: Result of organoleptic evaluation of herbal anti-aging cream

| Parameter | Anti-aging cream |
|-------------|-----------------------------|
| Color | Creamy yellow |
| Odour | Mild, pleasant herbal smell |
| Consistency | Good |
| Homogeneity | Good |
| Washability | Easily washable |

Table 4: Result of viscosity, pH, and spreadability of herbal anti-aging cream

| S. No. | Formulation | Viscosity (cP) | pH | Spreadability (cm) |
|--------|------------------|----------------|------|--------------------|
| 1. | Anti-aging Cream | 3992.4 | 5.02 | 7.00 |

4.3. Stability Study: To determine the stability of the herbal anti-aging cream, a stability study was conducted at three different temperature conditions. The temperatures used in the stability study were 25 °C, 37 °C, and 44 °C. The cream-maintained stability for four weeks at each temperature. Table 5 provides additional information.

Table 5: Stability studies of anti-aging cream

| Time duration | Temp. (°C) | Appearance | Homogeneity | Spreadability | After feel | Type of Smear | Removal | Irritation |
|----------------------|------------|--------------------|-------------|---------------|----------------------------|---------------|---------|------------|
| 1 st week | 25°C | No change in color | Good | Good | Emollient and slipperiness | Non-greasy | Easy | Nil |
| | 37°C | No change in color | Good | Good | Emollient and slipperiness | Non-greasy | Easy | Nil |
| | 44°C | No change in color | Good | Good | Emollient and slipperiness | Non-greasy | Easy | Nil |
| 2 nd week | 25°C | No change in color | Good | Good | Emollient and slipperiness | Non-greasy | Easy | Nil |
| | 37°C | No change in color | Good | Good | Emollient and slipperiness | Non-greasy | Easy | Nil |
| | 44°C | No change in color | Good | Good | Emollient and slipperiness | Non-greasy | Easy | Nil |
| 3 rd week | 25°C | No change in color | Good | Good | Emollient and slipperiness | Non-greasy | Easy | Nil |

| | | | | | | | | |
|----------------------------|------|--------------------|------|------|---------------------------|------------|------|-----|
| | 37°C | No change in color | Good | Good | Emollient and slipperines | Non-greasy | Easy | Nil |
| | 44°C | No change in color | Good | Good | Emollient and slipperines | Non-greasy | Easy | Nil |
| 4th week | 25°C | No change in color | Good | Good | Emollient and slipperines | Non-greasy | Easy | Nil |
| | 37°C | No change in color | Good | Good | Emollient and slipperines | Non-greasy | Easy | Nil |
| | 44°C | No change in color | Good | Good | Emollient and slipperines | Non-greasy | Easy | Nil |

5. CONCLUSION

A safe, effective, and stable natural anti-aging cream has been successfully developed. The cream incorporates ultrasound-assisted extracts of three species of flowers: butterfly pea, calendula, and chamomile. The extracts of the butterfly pea flower have the most potential for use in medicine. This new skin care product delivers great results while minimizing any potential adverse reactions.

ABBREVIATION

AlCl₃ – Aluminum chloride, BPF – Butterfly pea flower, °C- Degree Centigrade, CHCl₃ – Chloroform, cm – Centimeter, conc. – Concentration, dil. – Diluted, dis. – Distilled, DNA – Deoxyribonucleic acid, DPPH – 1, 1-diphenyl-2-picryl hydrazyl, FeCl₃ – Ferric chloride, H₂SO₄ – Sulphuric acid, HCl - Hydrogen chloride, g – Gram, M – Molar, mg – Milligram, ml – Milliliter, N – Normal, NaOH – Sodium hydroxide, ppt – Presentation, RSA - Radical scavenging activity, UV – Ultraviolet

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AUTHORS CONTRIBUTIONS

SS-Conceptualisation, reviewing and editing; LS, SS-Writing and reviewing; AD, SS- SS-Reviewing. All the authors agree to the publication of the manuscript.

Conflict of interest

All authors have approved the final manuscript, and the authors declare that they have no conflicts of interest to disclose

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