

Formulation and Evaluation of Polyherbal Anti-Aging Cream of Clitoria Ternatea, Mangifera Indica and Annona Squamosa

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Abstract: *The main objective of the present study is to formulate the polyherbal antiaging cream and evaluation of various parameters of the cream. The evaluation parameters consist of phytochemical evaluation, physiological evaluation, stability studies, homogeneity, appearance, ph, etc. The polyherbal cream was formulated on basis of antioxidant activity of selected plant extracts. The cream was formulated by using natural herbal ingredients like Clitoria Ternatea (Butterfly pea), Mangifera indica (mango), and Annona squamosa (Custard Apple). Extraction of Mangifera indica and Annona Squamosa was carried out by cold maceration where Ethanol and distilled water used as solvent (Hydroalcoholic extraction). Extraction of Clitoria Ternatea was carried out by hot water extraction by using distilled water as a solvent. Phytochemical screening all the three extracts shows presence of flavonoids, tannins, alkaloids, and phenols. Formulated cream was evaluated by using different parameters such as pH, Appearance, Spreadability, Washability, Irritancy test, Stability Studies, etc. There is no evidence of phase separation and final formulation do not show rashes or redness, edema on skin. These study was suggest that different composition of all three extracts and base used in the cream are more stable and safe. It can be concluded from present study that herbal cream having an antioxidant activity without any side effect and can be used as a provision to barrier of skin and to avoid skin aging.*

Keywords: Polyherbal Anti-aging cream, Skin aging, Clitoria Ternatea, Mangifera indica, Annona Squamosa, Antioxidant, Free radical.

I. INTRODUCTION

The skin is the main physical barrier to protect the body from the outside environment, skin also acts as a physical barrier to prevent the entry of foreign pathogens. It consists of the different layers such as: subcutaneous, epidermis, dermis. The disability of skin barrier function contributes to decrease in collagen content, stratum corneum hydration, skin phase out content, trans epidermal water loss. Aging is causes due to intrinsic and extrinsic factors. Intrinsic factor is part of genetic factor. Extrinsic factors having big concern with sun exposure, pollution poor nutrition which lead to nutrition, pigmentation and loss of smoothness, hydration. Free radicals are produced by the exposure to the ultraviolet rays by interaction with endogenous photo sensitizers which lead to cause alteration in the skin layers which affects the appearance of the skin. Free radicals are the main cause of many skin diseases, including skin cancer, wrinkles, aging. FR cause oxidative stress, the Antioxidative defines mechanisms are most effective to eliminate and diminish the action of them. Topical application of antioxidant safeguards the skin against environmental factors. Antioxidant compounds are the important key ingredient in skin caring products such as creams. Antioxidants such as, carotenoids, flavonoids, and tannins, alkaloids Vitamin E and C are present in various plants insubstantial amount which can be used to scavenge the overindulgence FR from the human body. There is no polyherbal formulation which developed though Mangifera indica, Clitoria Ternatea, Annona Squamosa reported for antioxidant properties. Hence, the present condition we are interested to formulate an antiaging cream using Clitoria Ternatea, Mangifera indica and Annona squamosal. It it observed that the process of skin rejuvenation can stimulated by different plant extracts content in biomarkers that is flavonoids, alkaloids, tannins, triterpenes and other biomolecules. Clitoria ternatea (family: Fabaceae) consist of anthocyanins and the structure of anthocyanins allows anthocyanins to display direct antioxidant activity toward radicals having two named

mechanisms: single electron transfer (SET) and hydrogen atom transfer (HAT). The chemical constituents present in the flowers of the *Clitoria Ternatea* show that they have antioxidant activity and some pharmacological evidence prove that the extract of *Clitoria Ternatea* flowers are used in cosmetics. *Annona squamosa* (family: Annonaceae) are contains flavonoids such as flavonoids the leaves of *Annona Squamosa* acerogenins, polyphenols are present which probably gives the effective FR scavenging activity.it works by increasing collagen and cellular proliferation. Mango (family: Anacardiaceae) leaves have been used in traditional medicine to treat anaemia, cutaneous infections, diabetes, diarrhoea, scabies, syphilis, and malignant tumours. The antioxidant activity of custard apple is Demonstrated by using various extracts. The glycaemic sugar content in the custard apple is low and it is suitable for diabetic patients Mango extracts have been characterized as exhibiting antioxidant, anti-inflammatory, analgesic, and immunomodulatory activities. According to Ayurveda, Various parts of *Mangifera indica* has been used to treat various disease and it also has an antioxidant activity. *Mangifera indica* L.(Anacardiaceae) is a medicinal plant of which Studies have also shown that some beneficial bioactivities of this plant include anti-cancer, anti- oxidant, anti-cancer antibacterial, anti-hyperglycaemic.

II. PLANT PROFILE

1. CLITORIA TERNATEA:

Synonym: Butterfly pea

Family: Fabaceae

Source: Dried Flowers of *Clitoria Ternatea*

Role: *Clitoria Ternatea* is a natural food-colorant containing anthocyanin, demonstrated antioxidant and antihyperglycemic activity. Marker compounds: -

Polyacylated derivatives of delphinidin 3,3',5'-triglucoside also known as "ternatins" are the major anthocyanins observed in blue pea flower. Traditional uses: -

- Butterfly pea traditionally are used to treat health issues such as indigestion, constipation, arthritis, liver and skin diseases, intestinal problems and as an antioxidant.
- The flowers of *C. Ternatea* are used traditionally used as a food colorant and worldwide as ornamental flowers.

2. MANGIFERA INDICA:

Synonyms: Mango

Family: Anacardiaceae

Source – Dried leaves of *Mangifera indica*

Role: *Mangifera indica* is one of most popular in all tropical fruits. *Mangifera*, being a glucose xanthine and polyphenolic antioxidant, it has majorly antioxidant.

Marker compounds: -

Flavonoids – Quercetin, Isoquercetrin, Gallic acid, *Mangifera*

Traditional uses: -

- Mango stem bark and leaves used in traditional medicine to treat diabetes, diarrhea, anemia, scabies, cutaneous infections and malignant tumors, syphilis.
- *Mangifera indica* extract characterized as exhibiting anti-inflammatory, antioxidant, and immunomodulatory activities, analgesic.

3. ANNONA SQUAMOSA:

Common name: - custard apple

Family: - Annonaceae

Source – Dried leaves of *Annona Squamosa*

Role: Extracts from custard apple leaves have been studied for their biological activities, including anticancer, antidiabetic, antioxidant, antimicrobial, anti-obesity, lipid-lowering, and hepatoprotective functions.

Marker compounds: -

Anonaine is major marker alkaloid observe in Hydroalcoholic leaves extract of the custard apple. It is majorly shown antioxidant activity.

Alkaloids – Anonaine

Flavonoids – Quercetin, Gallic acid, Kaemferol.

Traditional Uses: -

- Annona Squamosa leaves possess valorization potential owing to their extensive biological activities and pharmacological properties that is antioxidant, antimicrobial, antiviral, anticancer, antidiabetic and hepatoprotective activities

III. MATERIALS AND METHODS

1. CLITORIA TERNATEA:

Collection of Plant Material

The flowers of the Clitoria Ternatea were purchased online from flipkart in the month of March 2022. After collection of flowers they were shade dried and grinded into mortar and pestle to make coarse powder and stored in air tight container for further use.

Preparation of Extract

Previous studies on Clitoria Ternatea shows that Distilled water is the appropriate solvent for extracting anthocyanin from Clitoria Ternatea, because water could be considered as a non-flammable, nontoxic and inexpensive green solvent. Therefore, present study mainly focus on the extraction of anthocyanins from Clitoria Ternatea flowers with distilled water.

2. MANGIFERA INDICA

Collection of plant Material

The leaves of the Mangifera indica were collected from the local area of Rajuri of the district pune in the month of march 2022. After collection of leaves they were shade dried and made coarse powder of leaves by grinding in motar and pestle and stored in a closed air tight container for further use

Preparation of the Extract

The Grinded leaves powder of Mangifera indica were subject to the cold maceration by using ethanol and distilled water (70:30) as a solvent. After 72 hours filter the extract (Hydroalcoholic extract) and collect it.

3. ANNONA SQUAMOSA

Collection of Plant Material

The leaves of the Annona Squamosa were collected from the local area of Rajuri of the district pune in the month of march 2022. After collection of the leaves they were shade dried and made coarse powder of leaves by grinding in motar and pestle and stored in a closed air tight container for further use.

Preparation of the Extract

The grounded leaves powder of custard apple was subjected to the cold maceration by using ethanol and distilled water (70:30) as a solvent. After 72 hours filter the extract (Hydroalcoholic Extract) and collect it.

PREFORMULATION STUDY

Test for flavonoids

Shinoda test:

Take Conc. Hcl and some pieces of magnesium turnings and mixed it with crude drug extract, observe after few minutes, the development of pink, orange or red to purple colour indicates the presence of flavonoids.

Test for phenolic compounds & tannins

Fecl₃ test:

The alcoholic extract was taken in test tube and add 1% Fecl₃ solution, Formation of milky white colour after addition of fecl₃ indicates the presence of tannins and phenols.

Test for the steroid

Salkowski Test:

Take 2 ml of test extract and add 2 ml chloroform and 2 ml conc. sulphuric acid, Shake well the mixture, chloroform layer appears as red and acid layer will show greenish yellow colour.

Test for alkaloids

Wagner's test:

Take about 1 ml of extract and add to 2 ml of Wagner's reagent (Iodine in potassium iodide), Reddish brown precipitate indicates the presence of alkaloids.

Test for Carbohydrates

Molisch Test:

2 ml of sample + 2 drops of Molisch reagent and conc. Sulphuric acid slowly by sides, formation of purple ring indicates presence of carbohydrates.

FORMULATION OF THE CREAM

Table 2: Ingredients used in formulation of cream.

Sr. No.	Ingredients	Category	Formula % w/w
1.	Extract of M. Indica Leaves	Hydroalcoholic extract	0.5
2.	Extract of Custard apple Leaves	Hydroalcoholic extract	0.5
3.	Extract of Clitoria Ternatea flowers	Aqueous Extract	1.0
4.	Stearic acid	Emulsifier + Base	8
5.	Bees wax	Base	3
6.	Glycerine	Humectant	4
7.	Cetyl alcohol	Emollient	4
8.	Propyl paraben	Preservative	0.02
9.	Olive oil	Moisturiser	3
10.	Distilled water	Vehicle	Q.s

PROCEDURE FOR FORMULATION OF CREAM

PHASE I

It contains Ingredients like Cetyl alcohol, Stearic acid and olive oil are mixed using homogenisation and heated upto 75⁰c.

PHASE II

Contains all Water soluble components Propyl paraben, Glycerine, Aqueous Extract of Clitoria Ternatea and Hydroalcoholic extracts of Mangifera indica, and Annona Squamosa are mixed and heated upto 75⁰c until uniform consistency is formed. Phase I and phase II are mixed together at same temperature to form uniform mixture.

PHASE III

Phase III contains preservatives like propyl paraben is added to semisolid mixture at a temperature of 40⁰c. Stir the mixture by using mechanical stirrer and smooth cream was formed.

EVALUATION PARAMETER OF ANTI-AGING POLYHERBAL CREAM

A) Physico-chemical evaluation of Polyherbal Anti-aging cream

1. Evaluation of pH of the cream:

First calibration of pH meter is necessary then calibrate it with the help of standard buffer solution. Used 5 gm of cream quantity of this test and dissolve it in pure distilled water and then measure its pH.

2. Dye test:

Scarlet dye is used for dye test. Cream mixed with scarlet red dye. Put 1 drop of cream on slide and cover it by coverslip and observed under microscope. The cream is o/w type when red globules observe with Colourless background and The cream is w/o type when Colourless globules observe with red background.

3. Organoleptic Properties:

The organoleptic test of a cream preparation was done by visually including colour, odour, and clarity.

4. Irritancy test:

Highlight an area (1sq.cm on left hand dorsal surface). The cream was applied to the selected area and its time was noted. Erythema, irritancy, edema, was checked if any for regular intervals up to 24 hrs and reported.

5. Washability

Very less quantity of cream applies on hand and wash with the help of running tap water.

6. Spreadability

Formulation of cream placed between two glass slides and 100 gm quantity was placed on upper glass slide for 5 min and compress the formulation to uniform thickness. Weight 50gm was added to the pan. Separation of the two slides in seconds then measure its spreadability.

7. Homogeneity

All developed creams were tested for homogeneity by visual inspection after the creams have been set in the container. They were tested for presence of any aggregates and their appearance.

8. Appearance

The appearance of the cream is depending on its Pearlescence, color, and roughness and graded.

9. After Feel

Slipperiness, emolliency, and amount of the residue left after application of amount of cream were observe.

10. Type of Smear:

After application of cream on skin, the type of film or smear formed on the skin was checked.

11. Removal:

The easy of removal of cream applied were examined by washing the applied part with water.

B. Stability Study

Physical parameters like color, consistency and pH were determined at room temperature and 40°C.

Determination of Antioxidant Activity:

The FR scavenging activity of the H-donor ability was assessed using an ethanol solution of 2,2- diphenylpicrylhydrazyl (DPPH), a stable nitrogen-centered FR. FR scavenging activity of an anti- aging cream formulation was determined by DPPH which acts as a stable FR.

Sample Preparation:

About 10 mg cream formulations were weight and dissolve in ethanol. Prepared sample was filtered by the Whatman filter paper, and volume makes up to 10 ml in a volumetric flask.

Preparation of Standard:

Accurately weighed 10 mg of ascorbic acid and dissolved in 10 ml of ethanol. From this solution, take 2.5 ml and volume make up to 25 ml with ethanol thus the stock solution is prepared. This stock solution was serially diluted separately to obtain different concentrations.

Procedure:

Up to 3 ml of 0.004%, Ethanolic DPPH solution was added with the 0.5 ml sample solution. At 517 nm DPPH shows its absorbency and at the same wavelength sample absorbance was taken by a UV spectrophotometer after 30 min and then comparison was made between the absorbance of a sample and the absorbance of ascorbic acid (standard).

C. Anti-microbial Assay

Procedure

Using a modified agar well diffusion method, the antibacterial activity of several formulations was assessed. In this procedure, nutrient agar plates were seeded with 0.2 mL of Staphylococcus aureus/Candida albicans 24-hour broth culture. The agar plates were left to solidify. In each plate, a sterile 8 mm borer was used to cut two equidistance wells. Each Petri plate had a test solution in the first well and a standard solution in the second well, which were inserted at random. The plates were incubated for 24 hours at 37°C. The antibacterial activity was assessed by measuring the zones of inhibition (in mm).

Experimental conditions for anti-bacterial activity –

- Organisms used: Staphylococcus aureus
- Media used: Nutrient Agar.
- Test used: Polyherbal anti-aging cream.

Fig no 2 represent Antimicrobial assay of cream.

IV. RESULT AND DISCUSSION

Phytochemical Screening:

Phytochemical screening of all extract shows presence of flavonoids, steroids, tannins and phenols, alkaloids and carbohydrates are showed in table

Table 3: Phytochemical Screening

Phytoconstitute	Test	C .Ternatea	M .indica	Custard apple
Flavonoids	Shinoda test	+	+	+
Steroids	Salkowski test	+	+	–
Tannins & Phenols	Fecl3 test	+	+	+
Alkaloids	Wagner’s test	+	+	+
Carbohydrates	Molisch test	+	–	–



Fig no.1: Identification tests for C. Ternatea



Fig no.2: Identification tests for custard apple



Fig no.3: Identification tests for M. indica

Organoleptic Evaluation:

The morphological characteristics of the cream was evaluated for its colour, odour, texture and the results are given in the following table:

Table 4: Organoleptic Evaluation

Character	Observation
Colour	Blue
Odour	Characteristic
Texture	Smooth

Physicochemical Parameters:

Physicochemical Parameters like Ph, spreadability, dye test, washability, irritancy test, after feel, removal, homogeneity, type of smear, state were evaluated.

Table 5: Physicochemical Parameters

Sr.no.	Parameter	Observation
1.	PH	6.2
2.	Spreadability	Good
3.	Dye test	o/w
4.	Washability	Good
5.	Irritancy test	NIL
6.	After feel	Emollient
7.	Removal	Easy
8.	Homogeneity	Good
9.	Type of smear	Non greasy
10.	State	Semi solid

Stability Studies

Formulated cream was evaluated for stability study for period of three months. Table no 6 represent Stability study of cream

Table 6: Stability study of cream

S. No.	Parameter	Observation						
		Initial	First month		Second month		Third month	
			RT	40 °C	RT	40 °C	RT	40 °C
1	Appearance	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
2	Color	Blue	Blue	Blue	Blue	Blue	Blue	Blue
3	PH	6.2	6.2	6.4	6.2	6.4	6.2	6.1
4	Homogeneity	Good	Good	Satisfactory	Good	Satisfactory	Good	Satisfactory

Antioxidant Activity (DPPH):

The antioxidant activity of Polyherbal cream formulation was assessed using DPPH radical scavenging activity and by taking ascorbic acid as standard. Formulation shows maximum percentage inhibition of DPPH of about 80%, as compared to ascorbic acid which shows maximum 58% inhibition of DPPH.

Anti-microbial Assay

Table 7: Anti-microbial Assay.

Antimicrobial Agent	Formulation Code	Zone of Inhibition in mm
Staphylococcus Aureus	Polyherbal Anti-aging cream	15 mm



Fig no.4: Antimicrobial Assay

V. CONCLUSION

Due to constant exposure of human skin to the UV radiations present in sunlight causes several pathobiological alterations in cells occur such as increased wrinkling, irregular pigmentation, loss of elasticity and roughness, dryness. For the protection of this symptoms of aging herbal cosmetic are used as a therapy. Various active constituents such as phenolic acids and flavonoids appear efficient against UV radiation-induced damage the evaluation test reveals that the formulated anti-aging cream from Clitoria Ternatea, Mangifera indica and Annona Squamosa leaves extract showed that it is safe to be used in the skin to protect from intrinsic and extrinsic aging. Moreover, our research study presented that formulation is stable for 3 months. The formulation is homogeneous, emollient, non-greasy, and easily removed after the application and showed no evidence of phase separation, good spreadability and good consistency during the study period. Formulation shows the proper ph range which confirms the compatibility of the formulation with skin secretions. From this study, it is concluded that it is possible to develop anti-aging cream containing flower extract of Clitoria Ternatea and leaf extract of Mangifera indica and custard apple and it will help in reducing oxidative damage and give the antioxidant effect to our skin due to its high antioxidant values.

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CONFLICT OF INTEREST

The author declared no conflict of interest.

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