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Formulation Of Anti-Aging Cream by Using Natural Dye Pigment Anthocyanin

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Abstract
The major risk factor for all age-related disorders is ageing, a physiological process that is natural and unavoidable. Ageing poses a serious danger to people's health and creates a significant load on the public healthcare system. Thus, methods for extending life and preventing and treating disorders associated with ageing have drawn more and more attention from scientists. A subgroup of flavonoids called anthocyanin is abundantly present in *Clitoria ternatea*. Anthocyanins may help treat age-related disorders and slow down ageing. *Clitoria ternatea* dye pigments were screened for phytochemical analysis, antioxidant assay and UV spectroscopy. The Antiaging cream was formulated by using many components and purified anthocyanin pigments. Antibacterial activity was performed to know about the potential of the cream against the organisms.

Keywords: Aging, *Clitoria ternatea,* Anthocyanin, Antiaging cream, Antioxidant activity , Antibacterial activity

1. Introduction

A perennial herbaceous climber plant called *Clitoria ternatea* is also referred to as butterfly pea. It is a common tropical flower found in both gardens and the wild. *Clitoria ternatea* belongs to the kingdom Plantae, family Fabaceae, class Magnoliopsida, and phylum Tracheophyte. For human health and well-being, *C. ternatea* blooms' abundance of blue anthocyanins provides a number of benefits[1].

The *Clitoria ternatea* has many advantages it shows Antioxidant activity and also shows many Phytochemical active compounds[2,3]. Along with these properties, these flowers will also show anti-inflammatory which can be even more beneficial[4]. *Clitoria ternate*a L./blue pea flower is a rich source of polyacylated anthocyanins and their higher stability compared with non-acylated anthocyanins provide the advantage to be used as a natural food colouring agent[5].

Blue, red, or purple anthocyanins are pigments that are present in plants. The most typical places to find them are in flowers, fruits, and tubers. In acidic environments, anthocyanin appears as a red pigment, but in alkaline environments, it appears as a blue pigment. Despite possessing a positive charge at the oxygen atom of the C-ring of the fundamental flavonoid structure, anthocyanin is categorized as a flavonoid. The 2-phenylchromenylium ion is another name for it. The stability of anthocyanin is influenced by pH, light, temperature, and structure. Many plant blooms and fruits contain anthocyanins. The bulk of the red, purple, and blue blooms contained anthocyanins[6].

Anthocyanins could protect the aged skin induced by oxidant exposure as a major role in aging processing and skin degeneration[7].

Aging is an international epidemic that started some years ago with the emergence of life. The various harmful alterations brought on by aging that spread across cells and tissues gradually degrade function and may ultimately lead to death. Development, genetic flaws, the environment, illness, and the ageing process itself are all factors that influence how we age[8]. Anthocyanin's polyphenolic structure enables them to interact with metal ions, block the catalytic impact of active metal ions, reduce the production of free radicals, and have antioxidant properties. Anthocyanins may be used in anti-aging and anti-tumor therapies because of their antioxidant action, according to studies[9].

Creams are essentially emulsions and have a strong sense of value. The time for use in cosmeceuticals and pharmaceuticals has been extended by the stable creams created from W/O (water-in-oil) emulsion framework with a tall fluid stage supported[10]. A mixed preparation consisting of a water-in-oil emulsion-type moisturizing cream and a steroid ointment is frequently prescribed for the treatment of atopic dermatitis. As investigated the compatibility of moisturizing creams and ointments because there are concerns regarding the physical stability of these mixed preparations[11].

2. Materials and Methods

2.1 Sample Collection of Flowers

Clitoria ternatea flowers were collected from the streets of Coimbatore. The flowers were cleaned and petalswere collected and dried under the sun for 24 hours. Then the sample flowers were taken and stored. The dried petals were powdered using the blenders.

2.2 Extraction of Dye

10 grams of each powdered extract was taken and mixed with 100 ml of distilled water. All these were kept in a magnetic stirrer at 50°Celsius. The obtained samples were filtered by usingfilter paper and a funnel. The dye was extracted and stored in the refrigerator at 4 degrees Celsius.

2.3 Qualitative Analysis of Phytochemicals

The aqueous extract of *Clitoria ternatea* flowers was analyzed for the presence of various phytochemical constituents like tannins, alkaloids, flavonoids, saponins, terpenoids, glycosides, proteins, and other phytochemical compounds by standard protocol.

2.4 Antioxidant Activity by DPPH Assay

These antioxidants protect against damage caused by free radicals played important roles in thedevelopment of many chronic diseases including cardiovascular diseases, aging, heart disease,anemia, cancer and inflammation.

2.4.1Procedure

Preparation of DPPH-2,2-diphenyl-1-picrylhydrazyl

1M DPPH -4g DPPH and 100 ml of 99% methanol and keep it in cool conditions (Tris HCl buffer (pH-7.4).

The total free radical scavenging capacity of the extracts from the sample was estimated usingthe stable DPPH radical, which has an absorption maximum of 517 nm. A solution of the radical is prepared by dissolving 4 mg DPPH in 100 ml methanol. The aqueous extract of *Bougainvillea glabra, Clitoria ternatea,* and *Bougainvillea spectabilis* of 100 µl, 200 µl, 300 µl, 400 µl, and 500 µl was added to 3 ml of methanolic DPPH. The mixture was shaken vigorously and kept at room temperature for 30 min in the

dark. Absorbance of the reaction mixture was measured at 517 nm spectrophotometrically. Absorbance of the DPPH is radical without antioxidant, i.e. blank was also measured. All the determinations were performed in triplicate. The capability to scavenge the DPPH radical was calculated using the following equation. DPPH Scavenged (%) = ((AB–AA)/AB) ×100, where, AB is the absorbance of blankat t= 0 min; AA is the absorbance of the antioxidant at t= 30 min. A calibration curve was plotted with the % DPPH scavenged versus the concentration of standard antioxidants.

2.5 UV Spectroscopy

Different colored dyes vary in the wavelength of light that they absorb. Most dyes are conjugated compounds with alternating double and single bonds and typically absorb light in the visible region. The conjugated part of the dye molecule can be very short, meaning that there is a low degree of conjugation and few alternating double and single bonds, or long, meaning that there is a high degree of conjugation with many alternating double and single bonds**.**

2.5.1 Procedure

The dyes were observed in the UV Spectroscopy from the wavelength range of 500nm,520nm,540nm,560nm,580nm, and 600nm. The blank was kept as distilled water. The different sample was added to the cuvette and the readings were taken for the absorbance.

2.6 Column Chromatography

Column chromatography is a precursory technique used in the purification of compounds basedon their hydrophobicity or polarity. In this chromatography process, the molecule mixture is separated depending on its differentials partitioning between a stationary phase and a mobile phase. Column chromatography was performed to purify the dye and to obtain the anthocyaninextract from the dye which was prepared from *Clitoria ternatea*.

2.6.1Procedure

The column was prepared, and at the bottom sterilized cotton was placed upon which the silicagel 5ml was poured and the cotton was placed. 10ml of the dye should be poured into the column and the column was made to run for 6 hours. Fractions were collected.

2.7 Thin layer chromatography

Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminum foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used silica gel. On completion of the separation, each component appears as spots separated vertically. Each spot has a retention factor (Rf) expressed as:

R_f = distance traveled by the solute/distance traveled by the solvent

Thin-layer chromatography was performed to check the presence of the anthocyanin pigmentfrom the fractions of column chromatography.

2.7.1 Procedure

Butanol, Acetic acid and water was taken in the ratio of 4:1:5 the solvent was prepared. the TLC sheet was taken which is coated with silica gel and acts as the stationary phase. From the1cm above the TLC, the sheet line is drawn and the samples are spotted on the sheet. The sheetis made to run for 30 mins and RF values are calculated by using the formula.

Retardation Factor Value =Distance travelled by solute/Distance travelled by solvent.

2.8 Total Anthocyanin Content

Total anthocyanin content is used to determine how much amount of anthocyanin content ispresent in the fraction collected.

2.8.1 Procedure

A small liquate of the extract was diluted with the extracting solvent to provide an optical density within the instrument's optimum range. The diluted extract was stored in the dark for 4hours before being tested for absorbance at [λ]max 520 nm.

The total anthocyanin content was determined using the following equation

The total anthocyanin content (mg/100g) is calculated as [OD X DV X TEV X 100/SV X SWX 51.56].

Whereas, OD stands for optical density.

For the OD measurement, DV stands for diluted volume.TEV stands for total extract volume.

SV stands for sample volume.

SW stands for sample weight in grams.

51.56 = E. value of the main ingredient (Cyanidin).

2.9 Formulation of Base

Ingredients	Amount
Liquid Paraffin	1 ml
Bee wax	1.5 g
Tween 80	5 to 7 drops
Distilled water	16ml

The oil phase consisted of 1.5g of emulsifying beeswax and 1 ml of liquid paraffin was added and transferred into a 100 ml beaker and they were allowed to melt at 60ºC in a water bath. Using a glass rod to mix well. The aqueous phase 16ml of water was added and transferred intoa 100ml beaker constituting the aqueous phase. The aqueous phase was added to the oil phasegradually and kept in a magnetic stirrer at 50˚C.then 5 to 7 drops of Tween 80 were added andthen the cream was cooled.

2.10 Centrifugation test

The centrifugation test was performed to check whether the creams oil and liquid phase are mixed well together and to know whether the cream is stable or not.

2.10.1 Procedure

The prepared base was transferred into a centrifuge tube and was centrifuged at 5000rpm for 5minutes.

2.11 Formulation of cream

Ingredients	Amount
Liquid Paraffin	1 ml
Bee wax	1.5 g
Tween 80	5 to 7 drops

Distilled water	6ml
Anthocyanin extract	10ml

The oil phase consisted of 1.5g of emulsifying beeswax and 1 ml of liquid paraffin was added and transferred into a 100 ml beaker and they were allowed to melt at 60ºC in a water bath. Using a glass rod to mix well. The aqueous phase 6ml of water was added and transferred intoa 100ml beaker constituting the aqueous phase. The aqueous phase was added to the oil phasegradually and kept in a magnetic stirrer at 50˚C along with it 10ml of anthocyanin extract wasadded. Then 5 to 7 drops of Tween 80 were added and then the cream was cooled.

2.12 Evaluation of Anti-Aging Cream

1. **Physical Evaluation:** physical parameters such as color and appearance were checked visually.
2. **Measurement of pH:** The pH of various formulations was determined by using a Digital pH meter. One gram of cream was dissolved in 100ml of distilled water and stored for two hours. The measurement of the pH of each formulation was done in triplicate and with an average value.
3. **Stability study:** The stability study was carried out by storing the anti-acne cream at different temperatures are 4ºC and 27ºC.
4. **Homogeneity:** The formulation was tested for homogeneity by visual appearance and touch.
5. **After feel:** Emolliency, slipperiness and amount of residue left after the application of the fixed amount of cream was checked.
6. **Smear:** Smear was made on the skin.
7. **Removal:** The ease of removal of the cream applied was examined by washing the appliedwith tap water.

2.13 Antimicrobial Activity

Antimicrobial Susceptibility testing is used to determine which antimicrobials will inhibit the growth of the bacteria or fungi causing a specific infection. The results from this test will help a healthcare practitioner determine which drugs are likely to be most effective in treating a person's infection.

2.14 Procedure

Muller Hinton plates were prepared and gram-positive and gram-negative organisms (*S.aureus& E.coli*). A single streak was performed on the plates with the standard cream, test cream, andbase cream. The plates were incubated in the incubator for 24 hours.

3. Results and Discussion

3.1 Collection of Flower Samples

Fig 3.1 *Clitoria ternatea*

Fig 3.2 Powdered extract

Fig 3.3 Aqueous Extract by using Magnetic stirrer

Fig 3.4 Filtered Extract of Dye

3.2 Phytochemical Analysis

Fig 3.5 Phytochemical Analysis of *Clitoria ternatea*

Tannis	Absent
Alkaloids	Present
Saponins	Present
Steroids	Absent
Terpenoids	Present
Glycoside	Absent

Phenol	Absent
Quinone	Present
Proteins	Present
Carbohydrates	Present
Triterpenoids	Absent
Flavanoids	Present

Table 3.1 Phytochemical Analysis of *Clitoria ternatea*

3.3 Antioxidant Assay

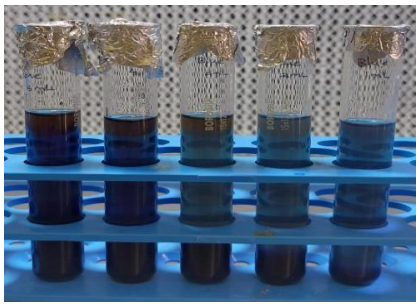

Fig 3.6 Antioxidant Activity of *Clitoria ternatea*

DPPH Assay

Concentration (*µL*)	**Control absorbance at 517nm**	**Sample absorbance at 517nm**	**%RSA**	**IC50**
100	1.05	0.916	12.7619	7.835448
200	1.05	0.832	20.7619	15.25605
300	1.05	0.758	27.80952	22.67665
400	1.05	0.581	44.66667	30.09725
500	1.05	0.334	68.19048	37.51785

Table 3.2 Antioxidant Activity of *Clitoria ternatea*

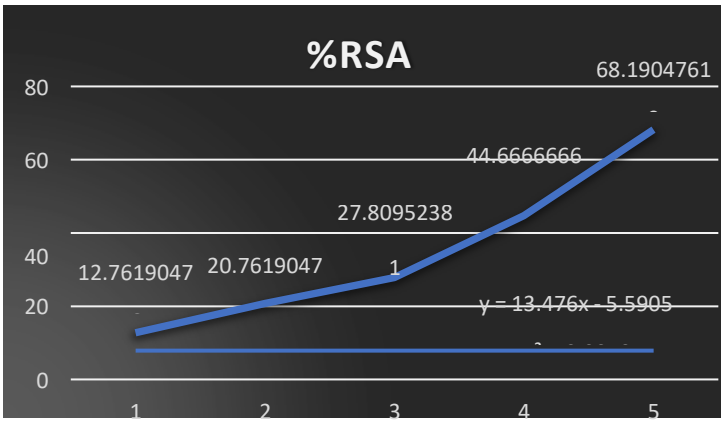

Fig 3.7 Graph for DPPH Assay

3.4 UV Spectroscopy

Wave length(nm)	Absorbance
500	0.257
520	0.353
540	0.456
560	0.615
580	0.785
600	0.721

Table 3.3 UV Spectroscopy of *Clitoria ternatea*

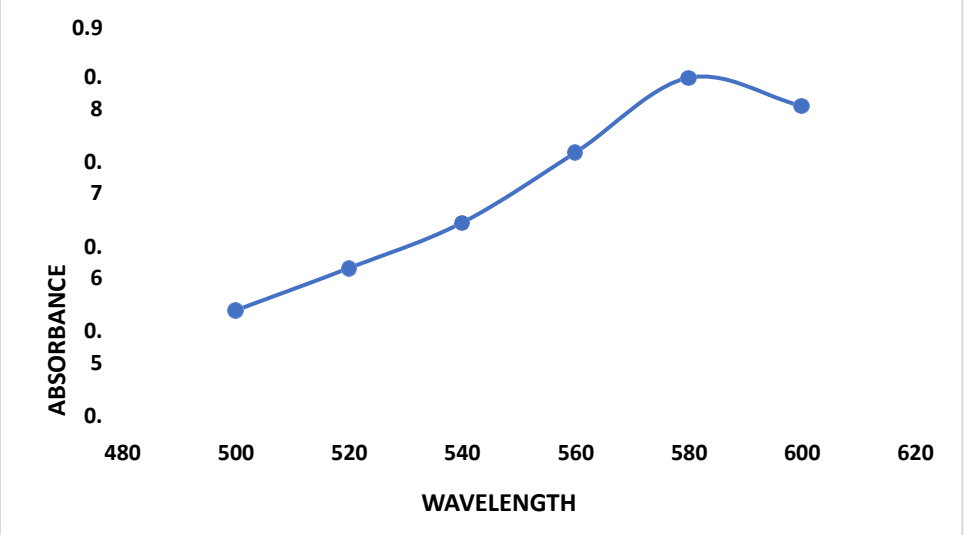

Fig 3.8 Graph for UV Spectroscopy of *Clitoria ternatea*

3.5 Column Chromatography

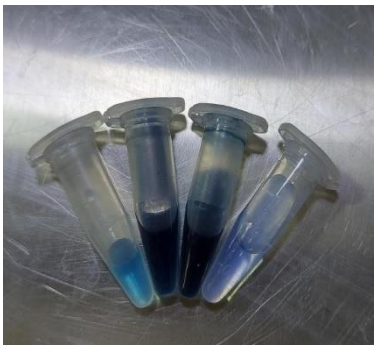

Fig 3.9 Purification of dye

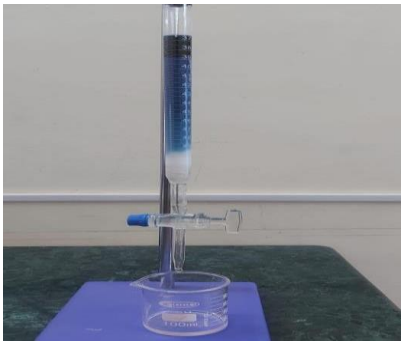

Fig 3.10 Fractions Collected

3.6 Thin Layer Chromatography

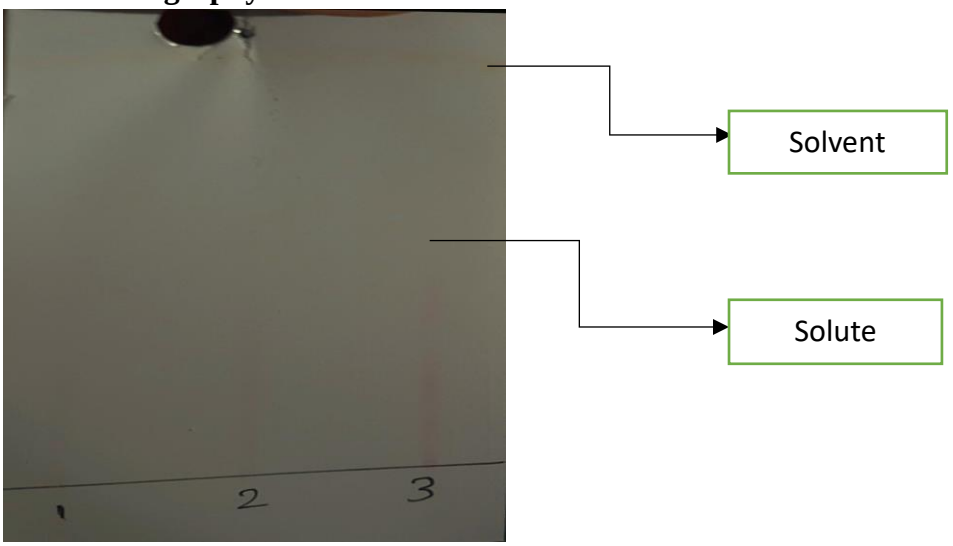

Fig 3.11 Confirmation of Anthocyanin by Thin Layer Chromatography

RF Value of Anthocyanin=0.32 -0.62RF Value=0.52

3.7 Total Anthocyanin Content

Fragment 2 OD value=0.26 & Total anthocyanin content=22.7
Fragment 3 OD value=0.99 Total anthocyanin content=86.4

3.8 Formulation of Base

Fig 3.12 Cream Formulation without Anthocyanin Pigment

3.9 Centrifugation Test

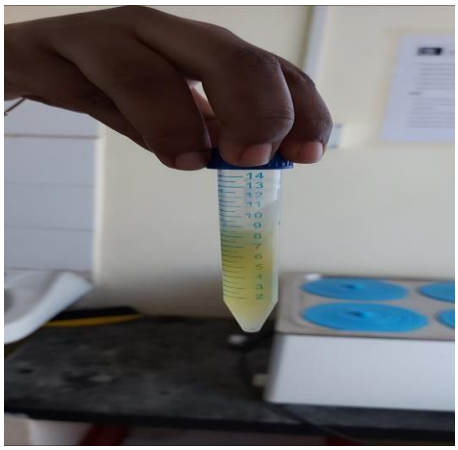

Fig 3.13 Cream was Stable by Centrifugation Test

3.10 Formulation of Cream

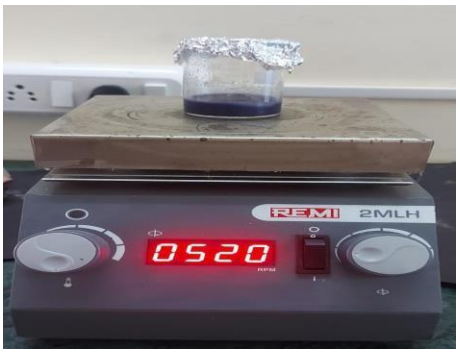

Fig 3.14 Cream Formulation by using Magnetic Stirrer

Fig 3.15 Final Cream was formulated with Anthocyanin Pigment

3.11 Evaluation of Formulated Anti-Aging Cream

S.No	Parameters	Cream
1	Appearance	Royal Blue
2	pH	6.5
3	Homogeneity	Good
4	Type of Smear	Greasy
5	After Feel	Emollien and Slipperiness

6	Removal	Easy

Table 3.4 Evaluation of Formulated Cream

3.12 Antibacterial Activity

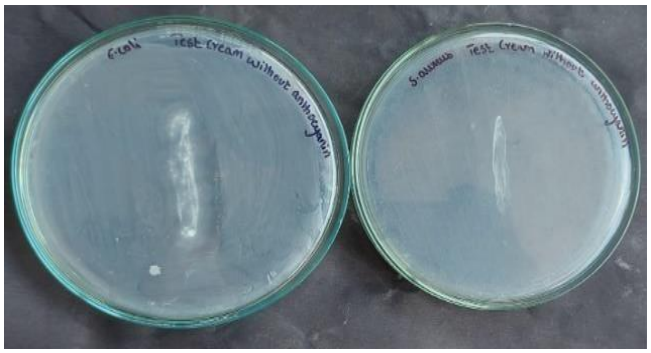

Fig 3.16 No Zones were Observed in Formulated Base Cream

Organisms	Zone of Inhibition
E.coli	No Zone
S.aureus	No Zone

Table 3.5 Antibacterial Test Results for Formulated Base Cream

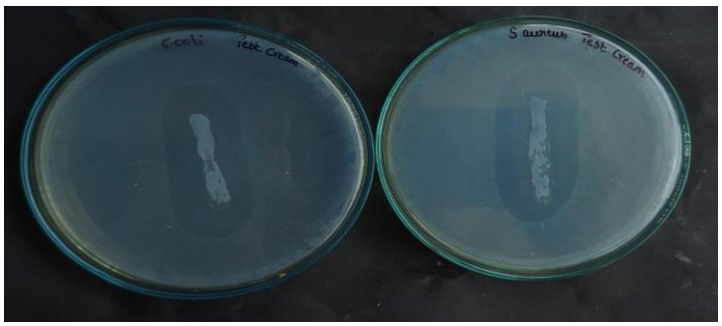

Fig 3.17 Zones were Observed in Formulated Cream with Anthocyanin

Organisms	Zone of Inhibition
E.coli	14mm
S.aureus	13mm

Table 3.6 Antibacterial Test Results for Formulated Cream

4. Summary and Conclusions

It has been discovered that the emulsions without oil are stable. The Water-in-oil emulsions have the potential to serve as a means of trapping anthocyanin and also as a promising mediumfor topical utilization. The emulsions containing anthocyanin have the potential to serve as a wholly natural moisturizing system due to their ability to absorb UV radiation, resulting in anincreased SPF value. Because they possess properties that act as antioxidants and reduce signs of aging, these emulsions are valuable additions to cosmetic formulas.

5. References

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