

Formulation And Evaluation of Anti-Ageing Gel Using *Clitoria Ternatea* Linn



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Abstract

The aim of the study is to develop an antiaging gel using *Clitoria ternatea* as the active ingredient, aiming to combat signs of aging on the skin. The project involves selecting and procuring high-quality, standardized plant material, conducting preformulation studies to ensure compatibility with the chosen gel base and excipients, and incorporating the extract into the gel base to ensure uniform distribution and optimal concentration. Physicochemical characterization tests will be conducted to evaluate the gel's pH, viscosity, appearance, and overall texture. Antioxidant activity evaluation will determine the extract's ability to combat oxidative stress, a common factor contributing to skin aging. The results from the formulation and evaluation process will contribute to advancements in skincare products and enrich the understanding of the benefits of herbal extracts in antiaging formulations.

Keywords: *Clitoria Ternatea* Linn; Hypodermis; Anti-ageing gel; cosmetics

Introduction

Skin is the major sign of age. With the onset of retirement age, there has been a rise in interest in anti-aging preparations, also known as cosmeceuticals, and their stated capacity to restore a person's young appearance. [1]. Anti-Aging Medicine is a type of preventative medicine that tries to enhance the quality of life (QOL) of elderly individuals [2]. Understanding the basic causes of human skin ageing has advanced substantially during the past decade. This knowledge serves as the foundation for the current use and future development of anti-aging medicines [3]. The optimal anti-aging intervention should be applicable for cosmetic preservation of an age-appropriate healthy appearance, preventing the onset of degenerative diseases, optimizing the function of the

Ageing brain and other tissues and acting on biological systems known to increase lifetime. Although there is ongoing controversy regarding the definition of anti-aging and the existence of a true anti-aging intervention, there is greater agreement regarding the elements that contribute to biologic ageing [4]. Collapsed fibroblasts in old skin generate low levels of collagen and high levels of collagen-degrading enzymes. This mismatch accelerates the ageing process in an unending, self-perpetuating cycle [5].

The quality of a person's skin can make all the difference in how old they appear. If your skin is leathery or wrinkled, you may appear ten years older than you actually are.

Similarly, smooth and supple skin can instantly remove years from your appearance. For this reason, it is essential to take care of your skin. For others, it's too late to take preventative measures, yet anti-aging skincare can perform miracles in turning back the clock [6].

Skin

The skin is the body's outermost tissue and largest organ in terms of both weight and surface area. It has a surface area of around 16,000 cm² for an adult and accounts for approximately 8% of body mass. The structure of skin is extremely complex and consists of numerous components. Multiple layers of cells, fibers, and other components give skin its multilayered structure. In this structure, veins, capillaries, and nerves form huge networks. In addition, hairs protrude from within the skin. Numerous fine hair furrows are dispersed across the skin's surface. As a result of chemical and physical reactions within these components, skin performs a vast array of activities. The primary purpose of skin is to serve as a barrier against the outer environment [7].

The skin produces a unique protective barrier against the external environment, regulating temperature and fluid balance, preventing the entry of hazardous microorganisms and chemicals, and protecting against the sun [8]. Due to external and internal factors, the collagen fibers and elastic fibers found in the dermal tissue of the skin are altered or destroyed, resulting in the creation of wrinkles and skin drooping because the skin's elasticity is diminished; this is one of the major reasons of skin ageing. Human skin is primarily constituted of three layers:

1. Epidermis
2. Dermis
3. Hypodermis.

Cosmetics

Cosmetics are described as substances that are intended to be rubbed, poured, sprinkled, or sprayed on, injected into, or otherwise applied to the human body or any portion thereof for the purposes of cleansing, beautifying, promoting attractiveness, or altering the appearance [9].

Anti-aging cosmetics

Anti-aging cosmetics are a subset of cosmetics that remove the effects of age and wrinkles on human skin. Anti-aging is an emerging class of cosmetics whose anti-aging chemicals decrease fine wrinkles and increase the skin's moisture content. The primary aim of anti-aging products is to diminish wrinkles and puffiness.

Gel

A gel is a solid or semisolid system of at least two constituents, consisting of a condensed mass enclosing and interpenetrated by a liquid. Gels and jellies are composed of small number of solids dispersed in relatively large amount of liquid, yet they possess more solid-like than liquid-like character. The characteristic of gel and jelly is the presence of some form of cutaneous structure, which provides solid-like properties [10]

Structure of gel

The rigidity of a gel arises from the presence of a network formed by the interlinking of particles gelling agent. The nature of the particles and the type of force that is responsible for the linkages, which determines the structure of the network and the properties of the gel. The individual particles of the hydrophilic colloid may consist of either spherical or an isometric aggregate of small molecules, or single macromolecules. Possible arrangements of such particles in a gel network. In linear macromolecules the network is comprised of entangled molecules, the point of contact between which may either be relatively small or consist of several molecules aligned in a crystalline order. The forces of attraction responsible for the linkage between gelling agent particles may range from strong primary valencies, as in silicic acid gels, to

weaker hydrogen bonds and Vander Waals forces [11].

Methodology

Evaluation Of *Clitoria ternatea* Extract

Characteristics of extracts

The physical state, colour, odour, of the ethanolic extract of *Clitoria ternatea* were mentioned in Table 1

Table 1: characteristics of the ethanolic extract.

Characteristics	Observation
Physical state	Semisolid
Color	Greenish brown
Odor	No odor

Phytochemical Investigation of the Extract

Phytochemical investigation of extract of *Clitoria ternatea* revealed the presence of flavonoids, phenolics and other constituents. Many of these compounds have been shown to produce potent anti-oxidant activity. The results were mentioned in Table 2 (Figures 1 & 2).

Table 2: characteristics of the aqueous extract.

Characteristics	Observation
Physical state	Semisolid
Colour	Purplish- blue
Odor	No odor

Optimization of Gelling Agent

Table 3 Different concentrations of carbomer-940 such as 1,1.5 and 2% were optimized to obtain gel with desired physical characteristics. Carbomer gel with 1% concentration shows good physicochemical properties for incorporating ethanolic extract of *clitoria ternatea* Figure 3

Formulation of Anti-ageing gel containing *clitoria ternatea*

Anti-ageing gel containing *clitoria ternatea* was incorporated into optimized. 1% Carbomer gel base. Different concentrations of ethanolic extract of *clitoria ternatea* such as 1,1.5 and 2% were incorporated in to Carbomer gel base Table 4.

Evaluation of Anti- Ageing Gel

Anti-oxidant Dpph Assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging:

$$\% \text{ DPPH inhibition} = \left[\frac{(\text{OD of control} - \text{OD of test})}{(\text{OD of control})} \right] \times 100$$



Figure 1: Result of Flavonoids test.



Figure 2: Result of Anthocyanins.

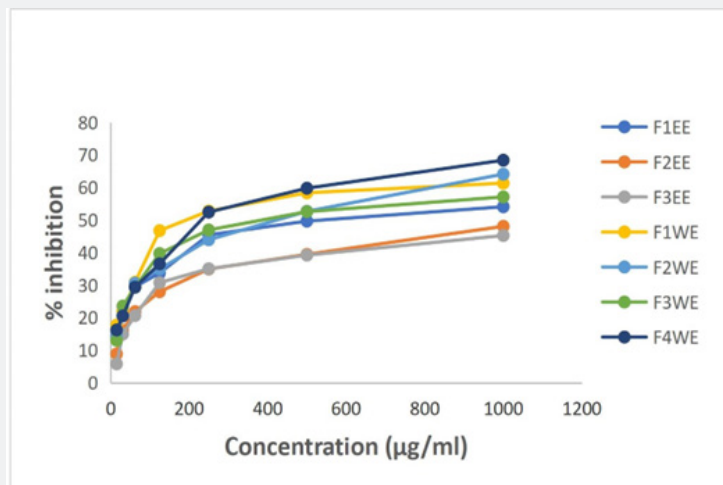


Figure 3: DPPH Assay Graph.

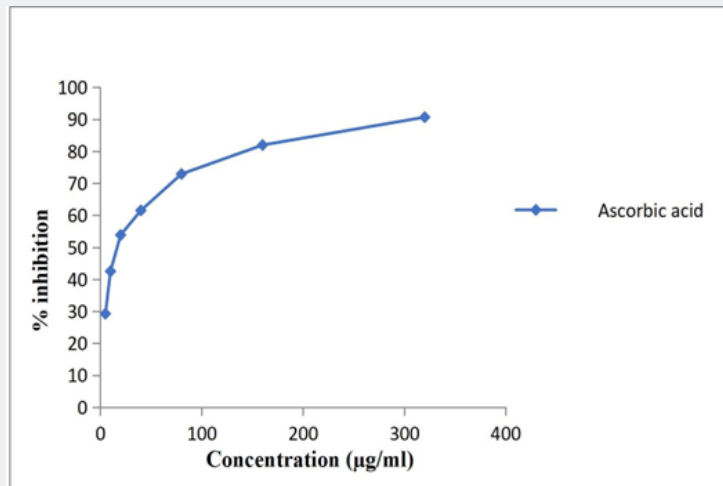


Figure 4: Control Sample Ascorbic Acid Assay.



Figure 5: Human volunteer no:1.



Figure 6: Human volunteer no:2.



Figure 7: Human volunteer no:3.

Table 3: Phytochemical investigation of the extract.

Constituents	Test	End point	Result
Flavanoids	Ferric chloride test	Green color	+
	Lead acetatesolution test	Yellow Precipitate	+
Tannins	gelatin test	White Precipitate	+
	NaOH test	Formation of emulsion	+
Anthocyanins	HCl Test	appearance of pink-red solution which turns blue-violet after addition of ammonia.	+
Glycosides	Ferric chloride test	formation of reddish brown colour at the junction of the two layers and the upper layer turned bluish green in the presence of glycosides.	+

Table 4: Anti-Oxidant Dpph Assay.

Test sample Conc.	F1EE	F2EE	F3EE	F1WE	F2WE	F3WE	F4WE
15.6	14.5428	8.85214	5.78798	17.7048	14.4946	13.0352	16.1965
31.2	21.9358	16.1476	14.9311	21.8876	20.5259	23.5406	20.5259
62.5	29.4741	21.8382	20.6227	30.8851	30.5939	29.3289	29.3773

125	33.7063	27.9663	30.7393	46.7415	34.9228	39.6882	36.5274
250	45.4282	34.9228	35.0196	52.7727	43.9203	46.9358	52.4311
500	49.6593	39.4453	39.2023	58.3656	52.6751	52.5772	59.7766
1000	54.0856	48.0547	45.2336	61.33268	64.1056	57.1017	68.3851
IC50 (µg/ml)	17.76	>1000	>1000	262.18	370.66	403.05	256.08

Table 5: Control sample assays.

Conc.(µg)	Ascorbic acid	%Inhibition	IC50 (µg/ml)
5	29.23152	29.23152	
10	42.50973	42.50973	
20	53.84241	53.84241	
40	61.52724	61.52724	
80	72.90856	72.90856	
160	81.95525	81.95525	
320	90.66148	90.66148	17.76

Table 6: Physical appearance of anti-ageing gel containing ethanol extract.

	F1	F2	F3
	[2.5%ethanolic extract of <i>Clitoria ternatea</i>]	[1.5% ethanolic extract of <i>Clitoria ternatea</i>]	[0.5% ethanolic extract of <i>Clitoria ternatea</i>]
Physical appearance	Transparent yellow gel	Transparent green gel	Transparent brown gel
Color	Pale yellow	Pale green	Brownish green
Homogeneity	Slight aggregates	Slight aggregates	Absence of aggregates

Table 7: Physical appearance of anti-ageing gel containing aqueous extract.

	F1	F2	F3	F4
	[2.5%water extract of <i>Clitoria ternatea</i>]	[1.5% water extract of <i>Clitoria ternatea</i>]	[0.5% water extract of <i>Clitoria ternatea</i>]	[0.5% water extract of <i>Clitoria ternatea</i>]
Physical appearance	Transparent y gel	Transparent blue gel	Transparent blue gel	Transparent blue gel
Color	Pale blue	Pale blue	Pale blue	Pale blue
Homogeneity	Slight aggregates	Slight aggregates	Absence of aggregates	Slight aggregates

Table 8: Measurement of pH.

Formulation Code	pH
F1WE	6.94
F2WE	6.87
F3WE	6.82
F4WE	6.79

Table 9: Determination of Viscosity.

Formulation code	Viscosity [cps]
F1WE	1340±0.50
F2WE	1358±0.25
F3WE	1425±0.75
F4WE	1428±0.10

Table 10: Spreadability.

Formulation code	Spreadability (gm.cm/sec)
F1WE	24.58
F2WE	22.35
F3WE	21.35
F4WE	19.37

Result

Figure 4 The IC50 value of the given test samples (F1EE, F2EE, F3EE, F1WE, F2WE, F3WE and F4WE) and reference standard ascorbic were found to be 547.02 µg/ml, >1000 µg/ml, >1000 µg/ml, 262.18 µg/ml, 370.66 µg/ml, 403.05 µg/ml, 256.08 µg/ml and 17.76 µg/ml, respectively Table 5.

Physical Appearance

The formulated gel was checked visually for color, appearance and homogeneity and the results were listed in Tables 6 & 7.

Measurement of Ph

The pH of all prepared formulation ranged from 5.5 -8.9. The pH of the prepared gel formulation was considered to be acceptable to avoid the risk of irritation upon application to the skin. The results were shown in Table 8

Determination of Viscosity

Viscosity is an important property of fluids which describes a liquid's resistance to flow and is related to the internal friction within the fluid. This rheological property helps in determining consistency and also the diffusion rate of drug from gel. The measurement of viscosity of the prepared gel was done with Brookfield viscometer with spindle no: 62. The results were shown in Table 9. By keeping the viscosity below about 15,000 cps, the advantages of more appealing cosmetic characteristics

and ease of accurate application through improved flow and pourability are achieved.

Spread Ability

Spread ability denotes the extent of area to which the gel readily spreads on application to skin. The spreading was expressed in terms of time in seconds taken by two slides to slip off from the gel, placed in between the slides, under certain load. Lesser the time taken for separation of the two slides, the better the spread ability. Two sets of glass slides of standard dimensions were taken. The gel formulation was placed over one of the slides. Spread ability of different gel formulation were studied. The formulation F1WE produced good spread ability than the other formulation. The results were shown in Table 10

Skin Irritation Test

Figure 5 The gel formulation was used for testing skin irritation. It was carried out on five human volunteers. The gel was applied in the hand of each volunteer few minutes. After removal of gel, the resultant skin effects were examined for the sign of erythema or itching. Figure 6 No erythema or edema was observed in the skin irritation test confirming that the gel was non-toxic and safe Figure 7.

Conclusion

Seven formulations were prepared using carbomer 1% gelling agent carbomer940 and they were evaluated for physical appearance, viscosity, spread ability and skin irritation test. Formulated gels were homogeneous, stable and showed better permeation.

All these investigations have brought out ultimate factors which leads to the following conclusions:

- i. The flower extract of *Clitoria ternatea* Linn. were incorporated in to optimized Carbomer gel base.
- ii. The data showed that the anti-ageing gel prepared from the extract of *Clitoria Ternatea* Linn. gave significant anti-oxidant activity when compared with standard ascorbic acid through DPPH assay.
- iii. As phytochemical test showed the presence of flavonoids, anthocyanin may produce an effect to minimize the ageing problem.
- iv. Among the formulation F1WE and F4WE (262.18 and 256.08 (IC50 µg/ml) showed better anti-oxidant activity.
- v. No erythema or edema was observed in the skin irritation test confirming the gel was non-toxic and safe.

Overall, this study reports concluded that the formulation of anti-ageing gel may offer an effective and safe dosage form which leads to patient adherence and compliance to the therapy as topical drug delivery.

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