



Fabrication And Characterization Of Face Serum Containing Sericin

¹DIONA M J, ²ARATHY S NATH, ³NIMMI MARIAM JOSE, ⁴ANSA ANDREW, ⁵ZADIYA ASLAM, DR. PRAVEEN RAJ R

¹Student, ²Student, ³Student, ⁴Student, ⁵Student, Professor : Department of pharmaceutics

¹St.Joseph'S College Of Pharmacy, Cherthala,

²St.Joseph'S College Of Pharmacy, Cherthala,

³St.Joseph'S College Of Pharmacy, Cherthala,

⁴St.Joseph'S College Of Pharmacy, Cherthala,

⁵St.Joseph'S College Of Pharmacy, Cherthala, St.Joseph'S College Of Pharmacy, Cherthala

ABSTRACT

The current demand for skincare products and treatments has significantly risen. There is a growing fascination among individuals to enhance their appearance. Consequently, a variety of products have been launched to promote a youthful and attractive look. Serums have emerged as a key component in establishing an effective skincare routine. They come in a variety of formulations suitable for different skin types, including oily, dry, and everything in between. In this study, we examine a face serum suitable for all skin types. The primary component of the serum is sericin, known for its antityrosinase, anti-oxidant, hydration and anti-aging properties. Additional ingredients include chamomile extract, hyaluronic acid, and vitamin E. Hyaluronic acid is a crucial element in cosmeceuticals due to its hydrating and anti-aging benefits. Vitamin E has demonstrated significant antioxidant and moisturizing benefits for the skin, offering protection against UVB radiation damage. Chamomile essential oils are widely utilized in the fields of cosmetics and aromatherapy. Currently, there is no existing treatment that can effectively slow the aging of the skin. However, serums containing a blend of concentrated vitamins and acids have demonstrated remarkable results. The face serum was assessed for its physicochemical properties, including pH, viscosity, antimicrobial activity etc. The increasing focus on skincare indicates a societal change that places greater importance on individual appearance and beauty ideals.

Key words: Face serum, sericin, antityrosinase, anti-oxidant, anti-aging, hyaluronic acid, chamomile.

INTRODUCTION

In the hustle and bustle of daily life, taking care of our skin often takes a backseat. However, maintaining a healthy, vibrant complexion requires a daily face-care routine. A face serum is a skin care product that is lightweight and extremely concentrated, able to permeate the skin deeply. Serums include a higher concentration of active substances, including vitamins, antioxidants, peptides, and proteins with just a few drops, these effective formulations treat particular skin issues and provide significant results. There are many different kinds of face serums that target problems like acne control, anti-aging, brightening, and hydration.

Sericin containing face serum, designed to rejuvenate and hydrate skin with the unique protein derived from silkworms. Sericin, known for its deep moisturizing and anti-aging properties, helps to form a protective barrier, locking in moisture and promoting a smooth, radiant complexion. Sericin also improve elasticity of skin, reduce fine lines, or simply enhance skin's natural glow by inhibiting the activity of tyrosinase enzyme and it also have antioxidant properties.

The silkworm *Bombyx mori* secretes sericin, a globular, gummy, water-soluble protein, to form sticky layers that encircle the fibroin, the primary silk fiber. The cocoon is created in this manner, holding the thin fibroin fibers together. About 20–30% of the overall weight of the cocoon is made up of sericin (1). Sericin is extracted from silk fiber in the textile industry and typically released with wastewater from silk processing. Because of their high organic content, these effluents seriously pollute the ecosystem in receiving water bodies (2). Nonetheless, sericin is a useful protein with a low cost that finds numerous applications in the pharmaceutical, biological, and cosmetics sectors. The purity of sericin affects its price on the international market.

TYPES OF FACE SERUM

i) Oil Serum

The oil serum is the easiest to create of all facial serums. The usual starting point is a mixture of high-quality, quickly-absorbing carrier oils, which are also known as 'dry' oils. In addition to providing moisturizing and barrier-repairing benefits, the premium oils in the serum also provide necessary fatty acids, polyphenols, and other substances that the skin can digest.



Figure 1: Oil serum

ii) Gel Serum

Gel serums create a tightening effect on the skin, causing it to feel uplifted or tighter in particular areas of the face. Due to the water-based nature of this formula, the gel serum allows for the incorporation of amazing water-based botanical extracts. Although gels are typically thought of as translucent, but can give them colour by adding glycerides such as colourful extracts and using vibrant botanicals.



Figure 2: Gel serum

iii) Water-based Serum

Water-based serums are comparable to gel serums, but they have fewer gums and thickeners. They can be used to deliver hydrophilic plant extracts of high performance that are trapped against the skin under a cream or lotion. The ideal method for promoting increased skin penetration of water-based compounds and delivering their high-performance. Applying an anti-aging face mist under an emulsion and then an oil will allow the ingredients to penetrate a little deeper into the skin layers. The oils will create a barrier that is occlusive, which will encourage the penetration of ingredients.



Figure 3: Water based serum

iv) Emulsion Serum

An emulsion-based face serum is a moisturizer that enhances the skin's barrier function and delivers high-performance results. By combining two "immiscible" phases, like oil and water, it uses an emulsifier to

bind them and keep them in a stable state. The best way to deliver high-performance actives into the skin's tissues is through this serum.



Figure 4: Emulsion Serum

v) Pressed Balm Serum

Balm serums are usually made up of butters, waxes, and oils, but they also contain active substances that are oil-soluble and can benefit the skin. By creating an occlusive barrier on the skin, the butters and waxes hydrate and nourish it, allowing the active components of the pressed serum to perform their duties. Combining thousands of exquisite plant oils with dozens of intriguing and unique butters and waxes is possible in a balm serum.



Figure 5: Pressed balm serum

FACE SERUM CONTAINING SERICIN

Nowadays, different types of face serums are available in market. The demand for face serum is increasing day by day as it has several advantages over other formulations such as creams, lotions etc. When compared

to creams, face serums are more concentrated, absorbed through the skin rapidly and also has site specific action. Sericin containing cosmetic products are available in market but its combination with chamomile is rare. In this formulation we use combination of sericin-hyaluronic acid-chamomile essential oil that enhances the properties like:

- Antioxidant
- Antityrosinase
- Hydration
- Antiaging
- Anti-inflammatory
- Wound healing



Figure 6: Face serum contain sericin formulation

Vitamin E is used in this formulation which has Antioxidant activity. This formulation has a combination of sericin-hyluronic acid-chammomile essential oil. Vitamin E which increases the activity of reducing scars acne, hyperpigmentation and redness.

This formulation is a sericin containing water-based face serum and so can be used for any skin type as it reduces the breakouts and acne compared to oil base face serums. Almost all the cosmetics available contain PFAS (poly fluoroalkylated substance) and phthalates, which is toxic that can cause infertility, cancer, neonatal death etc. In this project we focused to develop a product free from PFAS and phthalates.

➤ HOW IT SHOWS ANTIOXIDANT PROPERTY?

The presence of elevated levels of hydroxyl amino acids makes silk sericin a potent natural antioxidant. Skin cells are constantly subjected to oxygen and frequently encounter solar radiation. This exposure leads to an increase in reactive oxygen species (ROS), which in turn raises the risk of developing skin cancer. Zhaorigetu et al. (2003) and Dash et al. (2008) explored the potential function of sericin in regulating

oxidative stress within epidermal cells. They conducted in vitro assay by using hydrogen peroxide. The analysis of sericin's impact on hydrogen peroxide-induced DNA damage in feline fibroblasts revealed an enhancement in cellular viability among the cells treated with sericin. Additionally, a reduced release of catalase (catalase: - is a protein, made up of amino acids), the enzyme responsible for breaking down hydrogen peroxide, was observed in the sericin sample. The results indicate that sericin protein may offer a protective benefit against oxidative stress. The influence of antioxidant effect also effects the method of extracting of sericin. Sericin extracted by the autoclaving or by using steam shows better protection.

DOES IT HYDRATE THE SKIN?

The stratum corneum of the skin should maintain a water content of 10 to 20% to ensure the skin remains plump and elastic. However, when the water level in the stratum corneum drops below 10%, the skin becomes dry and cracked. This outer layer of the skin contains a natural moisturizing factor (NMF) that is crucial for hydration, helping to retain the right moisture levels and making the skin smoother, softer, and more elastic. The sericin protein found in silk's outer layer serves as an excellent natural moisturizing factor. Its amino acid profile closely resembles that of the skin's NMF, and many polar groups in the sericin polypeptide chains are accessible on the surface. Water from the body can bind to the stratum corneum, helping to sustain moisture on the skin's surface. The moisturizing and hygroscopic properties of sericin are linked to its molecular structure. The soluble sericin primarily adopts a coil structure, and its polypeptide chains are in a loose and disordered arrangement. This configuration facilitates the accessibility of numerous hydrophilic groups on the surface of the molecule's spatial structure.

➤ DOES IT ENHANCE YOUR SKIN'S GLOW?

The development of melanocytes within the cortical layer can influence the lightening of human skin, while these melanocytes can also generate melanin, resulting in skin darkening. The melanin production in the skin occurs when melanocytes contain tyrosine, which causes the oxidation of tyrosine found in skin keratin proteins into melanin. However, UV radiation can enhance this oxidation process, making it more likely for skin to darken with frequent sunlight exposure during the summer months. When 1% sericin is introduced, the activity of tyrosine is reduced by over half, with a melanin inhibition rate of 52.39%. Sericin is composed of hydroxyl amino acids (serine, threonine) and trace minerals (copper, iron, etc.), and these components interact in complex ways. Consequently, this affects the normal functioning of tyrosine activity. Therefore, sericin effectively inhibits tyrosine activity, making it a powerful agent for reducing melanin formation in the skin. As a result, skin care products containing sericin can help maintain a fair and flawless complexion.

➤ HOW DOES IT SHOW ANTI-AGING PROPERTIES?

The studies indicate that the anti-aging effects of sericin are attributed to its ability to stimulate the synthesis of collagen type 1 and to decrease apoptosis, similar to the effects of vitamin C. However, Silk sericin exhibits a greater efficacy than vitamin C in promoting collagen synthesis. The anti-aging effects were

similar to those of vitamin C, with the exception of oxidative stress, in which silk sericin demonstrated greater efficacy.

Environmental and Economical Benefits

1. In the textile sector, sericin is extracted from the fibres and is typically released alongside wastewater generated from silk processing. The effluents lead to significant environmental contamination in the water bodies they enter because of their high organic content. By utilizing the sericin from the wastewater, we can prevent pollution.
2. Sericin is an important protein with economic significance, and it finds numerous applications in the cosmetics, pharmaceutical, and biomedical sectors. The market price of sericin worldwide fluctuates based on its purity and intended use. For instance, sericin that is appropriate for the cosmetic sector ranges from 40 to 120 euros per kilogram. In contrast, highly pure sericin that is used for cell culture research can reach prices up to 70 euros per kilogram.

Storage guidelines for Sericin containing face serum

This face serum is sensitive to light due to the presence of hyaluronic acid and chamomile extract. It is important to store it in amber coloured bottle for its optimal performance and longevity.

1. Cool and dry environment
2. Avoid direct sunlight
3. Temperature range

Method of using Sericin containing face serum

Sericin face serum is a vital water-based skincare product designed to address various skin issues. For best results, start by cleansing your face and gently patting it until it's slightly dry. When your skin is still moist, it's ideal to apply serums, which usually have a higher concentration of active ingredients. At this point, the skin is more receptive, enhancing absorption. Apply the serum using upward sweeping motions on your face. Serums should be applied in gentle tapping movements using your fingertips or palms. Avoid excessive rubbing of your skin; instead, allow the serum to absorb naturally.

INGREDIENT PROFILE

1. SERICIN

Sericulture is a complex and ancient practice that has played a significant role in the global textile industry and cultural heritage. This art of sericulture involves rearing of silkworms, the collection of their cocoons, and the extraction of silk fibers. Silk is a natural fiber that is generated by the silkworm species, *Bombyx mori* belonging to family bombycidae. The silkworm is a Lepidoptera insect which has the ability to produce silk and serves as the foundation of the silk industry. Mulberry leaves are the only source of nutrition for silkworm throughout its larva life. The larvae of the fifth instar spins a silk cocoon which consist of silk fibre and sericin binder.



Figure 7: Silk cocoon



Figure 8: Silk worm (*Bombyx mori*)

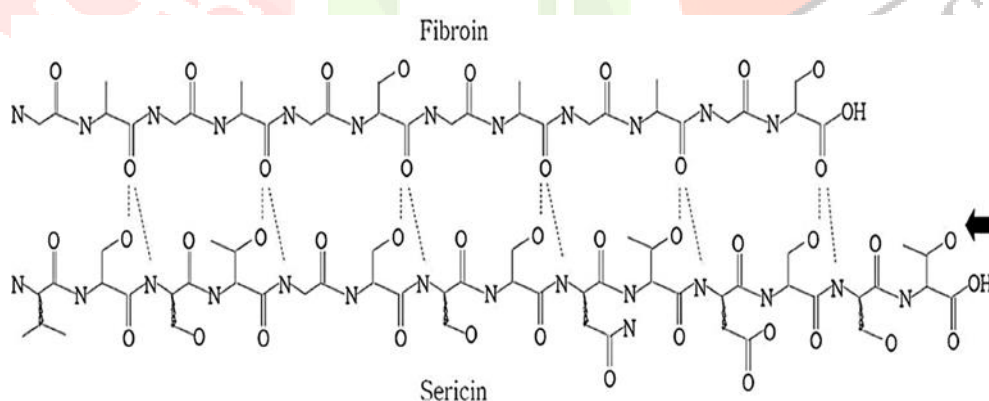
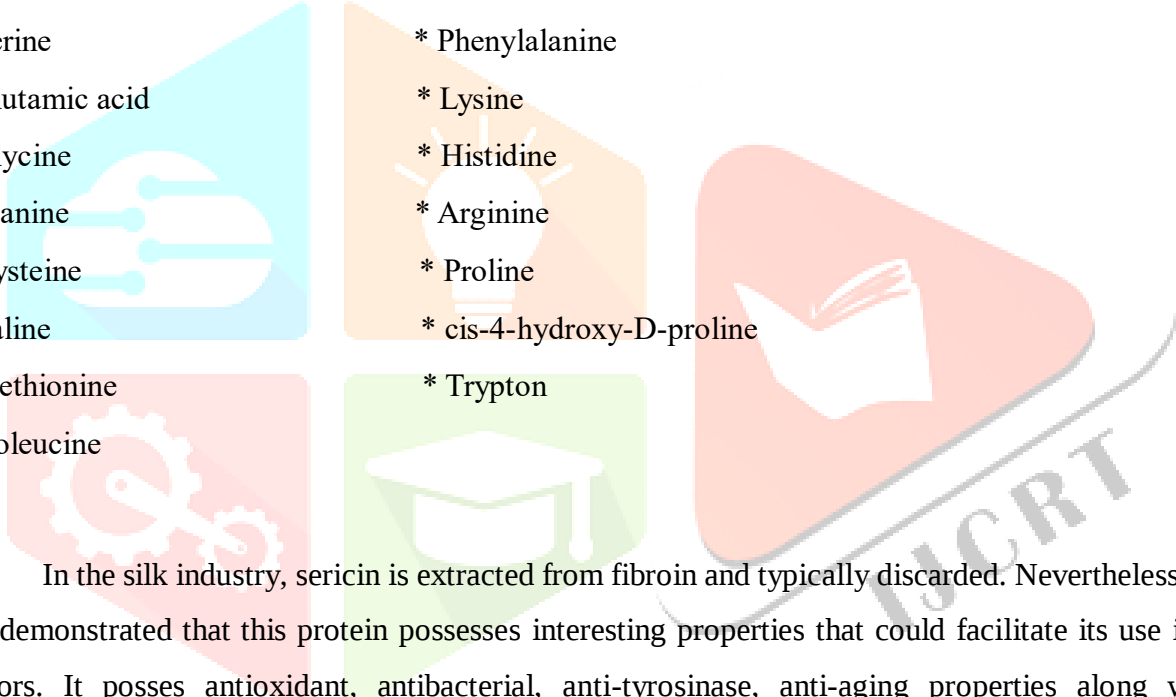


Figure 9: Structure of sericin

Silk is a protein fiber made up of two main components: sericin and fibroin. Fibroin accounts for about 70% to 80% of the composition of silk fibers. It features both amorphous and crystalline regions, characterized by short amino acid chains that contribute to its compact structure. The globular protein sericin, constitute approximately 20-30% of silk fiber and serves the purpose of coating and binding the fibroin filaments within the cocoons of silkworms. Sericin protein is composed of 18 different amino acids, the majority of which are classified as polar and serine and aspartine constitute about 33.4% and 16.7% of the total composition sericin, respectively. Sericin is structured in three distinct layers: outer, middle, and inner. The outer layer exhibits the highest solubility, while the layer that is in contact with fibroin can only be extracted through the application of high pressure, elevated temperatures, or alkaline substances.

List of Aminoacids present in Silk Sericin

- 
- * Aspartic acid
 - * Threonine
 - * Serine
 - * Glutamic acid
 - * Glycine
 - * Alanine
 - * Cysteine
 - * Valine
 - * Methionine
 - * Isoleucine
 - * Leucine
 - * Tyrosine
 - * Phenylalanine
 - * Lysine
 - * Histidine
 - * Arginine
 - * Proline
 - * cis-4-hydroxy-D-proline
 - * Trypton

In the silk industry, sericin is extracted from fibroin and typically discarded. Nevertheless, research has demonstrated that this protein possesses interesting properties that could facilitate its use in various sectors. It posses antioxidant, antibacterial, anti-tyrosinase, anti-aging properties along with anti-inflammatory property. The anti-aging benefits of sericin are mainly associated with the protein serine and anti-oxidant property is exhibited by cysteine. Sericin rich in serine amino acid enables it to bind to the active site of tyrosinase, obstructing the enzyme's role in melanin synthesis. Its moisturizing and anti-wrinkle characteristics make sericin a valuable ingredient for the cosmetic industry.

2. HYALURONIC ACID

The molecular structure of hyaluronic acid (HA) or hyaluronan is related to the glycosaminoglycans of mucopolysaccharides. In the skin, articular cartilage, synovial fluid, vitreous humor, and mammalian bone marrow, HA is found as a natural component. In soft connective tissues like skin, lungs, kidneys, brain, and muscles, there is a high amount of HA. Due to its distinctive viscoelastic properties, biocompatibility, and non-immunogenicity, it makes a great choice for both medical and cosmetic applications.

In the medical and cosmetic industries, this versatile substance has many uses, including dermal fillers, osteoarthritis treatment, ophthalmology and vesicoureteral reflux. The composition of this



Figure 10: Hyaluronic acid

glycosaminoglycan is high molecular weight, with glucuronic acid and N-acetylglucosamine as its constituents, with glycosidic bonds forming a bonding mechanism. HA has numerous and significant biological functions. It is vital for regulating tissue hydration and water transportation, keeping the elasto-viscosity of connective tissues, and facilitating the assembly of proteoglycans in the extracellular matrix by means of supramolecular interaction. Hyaluronic acid's anionic properties make it capable of attracting water to swell, creating volume, and providing structural support. As age increases, the amount of hyaluronic acid and collagen in the skin decreases. Wrinkles begin to form on top of the skin once its viscoelasticity is lost. When HA is bonded to water molecules, it forms a hydrated gel that serves as a water-binding agent that provides lubrication for movable body parts, including joints and muscles. Hyaluronic acid injectables are being used after botox in non-surgical cosmetic procedures due to changing beauty standards and rising health awareness.

3. CHAMOMILE EXTRACT

Chamomile, which is known for being one of the oldest aromatic plants, has been proven to have both anti-inflammatory and anti-aging effects through its flower and essential oils. It falls under the Asteraceae family and can either be grown as an annual or perennial plant.

The plant is effective in acne, sunburn and also reduces skin redness. Chamomile originates from temperate regions of Asia and Europe and is grown globally as a medicinal, cosmetic, and food ingredient in southern and eastern Europe, northern Africa, central and western Asia and western North America. Flavonoids (35.1%), coumarins, volatile oils (2%), terpenes, sterols, organic acids, and polysaccharides are the chemical constituents found in chamomile.

Apart from the cosmetic use chamomile also exhibit pharmacological activity such as anticancer,



Figure 11: Chamomile flower



Figure 12: Chamomile extract

anti-infective, antioxidant, hypoglycaemic, hypolipidaemic, antiallergic, antidepressant, neuroprotective effects. Perfume, skincare products, massage oil, and toothpaste are all made using the essential oil from this plant.

4. VITAMIN E

The cell antioxidant defence system is largely dependent on vitamin E, which is only obtained from the diet.

Due to its antioxidant activity, it plays a significant role in the body. Vitamin E is becoming increasingly popular in skin care due to its essential and beneficial role in various health aspects. Vitamin E is categorized into two categories: tocopherols and tocotrienols, each consisting of four isomers (alpha, beta, gamma, and delta). Many cosmetic products contain it as an essential ingredient. The skin is protected from detrimental effects caused by solar radiation as it acts as a free-radical scavenger. Vitamin E is commonly used to treat burns, surgical scars, and wounds. Vitamin E can stop serum lipid peroxidation caused by bacteria leaking through follicles and sebaceous glands and resulting in inflammation from peroxide irritation. Vitamin E is included in many over-the-counter products designed to treat skin aging.



Figure 13:Vitamin E

MATERIALS AND METHODS

The materials used in the preparation of serum are listed below:

MATERIALS USED

SL NO:	MATERIALS
1	Sericin
2	Hyaluronic acid
3	Vitamin E oil
4	Chamomile extract
5	Sodium carboxymethyl cellulose (CMC)
6	Sodium Benzoate

Essential ingredients of face serum containing sericin

1. Sericin:

Sericin is a protein produced by Bombyx mori, commonly known as silkworm, during the silk making process. It has moisturizing, anti-aging, antityrosinase property.

2. Hyaluronic acid:

Hyaluronic acid may assist in diminishing the visibility of wrinkles and fine lines, while also aiding in the prevention of clogged pores. It possesses characteristics such as hydration, anti-aging effects, wound healing capabilities and the ability to address facial redness and eczema.

3. Vitamin E:

It has properties such as antioxidant, hyperpigmentation, wound healing and also improve skin elasticity. Vitamin E reduces acne scars and swelling.

4. Chamomile extract:

Chamomile possesses various beneficial properties including skin lightening, soothing effects, antioxidant capabilities and antibacterial qualities. Additionally, it helps to diminish redness, irritation, acne and dark circles.

5. Sodium CMC:

It is used as a thickener and emulsifier.

6. Sodium Benzoate:

It is used as a preservative.

Method

I. Collection of materials from various sources

Silkworm cocoons were collected and thoroughly cleaned. All other ingredients were collected from various sources.



Figure 14: Silkworm cocoons collected from waynad



Figure 15: Cleaning of cocoons

II. Extraction of sericin

Cocoons were cut into small pieces. Clean and sterilized conical flask was taken, into which the cocoons were placed along with water in 1:10 ratio (3g in 30 ml). This mixture was then subjected to autoclaving for 20 minutes at a temperature of 121 degree Celsius and a pressure of 15 psi. Upon completion of the autoclave cycle, the conical flask was removed and the mixture was filtered using whatman filter paper to obtain the sericin extract.



Figure 16: Extraction of cocoons by Autoclaving



Figure 17: Three ratios of cocoons for extraction



Figure 18: Extracted sericin

III. Biochemical tests

- A) **Tannin precipitation test** :- 2ml of sericin solution was taken in a test tube. To this 1ml of tannic acid was added. Brown precipitate was formed.



Figure 19: Tannin precipitation test

- B) **Ninhydrin test** :- 3ml of sericin solution was taken in a test tube. To this 1 ml of ninhydrin reagent was added. Purple colour was obtained which indicate the presence of amino acid.



Figure 20: Ninhydrin test

- C) **Biurette test** :- 2ml of sericin solution was taken in a test tube. To this 1ml of biurette reagent (sodium hydroxide, copper sulphate, potassium sodium tartrate) was added. Violet colour was observed which shows the presence of protein.



Figure 21: Biurette test

WORKING FORMULAS FOR THE THREE FORMULATIONS

SL. NO	INGREDIENTS	F1	F2	F3
1	Sericin	*1:25 20 ml	*1:10 20 ml	*1:50 20 ml
2	Hyaluronic acid	6 ml	6 ml	6 ml
3	Vitamin E	0.5 ml	0.5 ml	0.5 ml
4	Chamomile extract	0.5 ml	0.5 ml	0.5 ml
5	Sodium carboxymethyl cellulose	2 ml	2 ml	2 ml
6	Sodium benzoate	1ml	1 ml	1 ml

*1:10 - 1g of coccon in 10 ml of distilled water.

1:25- 1g of coccon in 25 ml of distilled water.

1: 50- 1g of coccon in 50 ml of distilled water.

Method of Preparation:

- Sericin extract was placed in a beaker, which is positioned beneath a sanitized impeller for mixing purpose.
- Vitamin E was incorporated gradually, drop by drop, and then thoroughly mixed.
- Following this chamomile extract was also added drop by drop.
- Hyaluronic acid was introduced into a beaker along with an appropriate amount of water to create a gel consistency, which was subsequently combined with the previous mixture.
- Sodium CMC was dissolved in 10ml of ethanol (60%) and water mixture, filtered and added to the mixture.
- Additionally, sodium benzoate was dissolved in 10ml of water and 1ml of this solution was incorporated into the mixture, ensuring thorough mixing.



Figure 22: Mixing of face serum by using propeller

STORAGE:

The formulation was transferred into an amber coloured bottle and stored at room temperature.

EVALUATION

1. Physical Evaluation

Physical assessment of the formulation was conducted. The colour, odour, texture and overall appearance were examined.

2. Homogeneity

The serum sample is transferred into an airtight glass container and examined visually and by touch for the presence of particles. The serum is considered homogeneous if no visible particles are detected.

3. Skin irritation test

The skin irritation test involves applying the serum to the skin and observing for any signs of redness or itching after a duration of two hours.

4. pH Value

Standard buffer solutions with pH 4 and 7 are used for calibration. The pH meter was adjusted to these specific values to ensure accurate measurements. Sample of 0.5g was dissolved in 50 ml of distilled water and allowed to sit for 2 hours. Subsequently, the pH of the sample was determined using the calibrated pH meter.

5. Spreadability

Spreadability can be assessed using a device called the spreadability apparatus, which consists of a wooden board equipped with a scale and two glass slides having two pans on both sides mounted on a pulley. An excess quantity of the sample is placed between two glass slides and a 100g weight is placed on the glass slide for duration of 5 minutes to ensure the sample is compressed to a uniform thickness. A weight of 250 g is subsequently placed on the pan. The duration, measured in seconds, needed to separate the two glass slides was recorded as an indicator of spreadability.

Spreadability can be determined by using following formula:

$$S = m \times \frac{l}{t}$$

where,

S = Sample spreadability

m = upper plate mass (g)

l = glass plate length (cm)

t = time taken to separate (s)

6. Cyclical Temperature

This test is not conducted at a constant temperature and humidity. Instead, the temperature is varied cyclically each day, alternating between room temperature and freezing conditions to induce temperature fluctuations.

7. Anti-oxidant Test (FRAP Assay)

Transfer 2 ml of the sample solution into a test tube, followed by the addition of 2.5 ml of phosphate buffer solution. Mix the contents of the test tube thoroughly. Introduce 2.5 ml of a 1% potassium ferric cyanide solution to the mixture. Vortex the reaction mixture using a vortex shaker to ensure proper mixing. Incubate the mixture at 50 degrees Celsius for approximately 20 minutes. After the incubation period, add 2.5 ml of 10% trichloroacetic acid to the mixture. Centrifuge the test tubes at 3000 rpm for 10 minutes. From the resulting centrifuged sample, collect 2.5 ml of the supernatant into separate test tubes. To each of these new test tubes containing 2.5 ml of supernatant, add 2.5 ml of deionized water, followed by the addition of 5 ml of ferric chloride.

8. Viscosity

Brookfield viscometer was used to determine the viscosity of the formulation. Utilizing a spindle type model S63, the viscosity was measured at a speed of 100 rpm.

The spindle was immersed in 100 ml of serum in a beaker for about 5 minutes and readings were obtained.

9. Antimicrobial Assay

Anti-bacterial activity by Agar well diffusion method

In order to evaluate the antimicrobial activity of plant or microbial extracts, the agar well diffusion method is frequently used. The surface of the agar plate is inoculated utilizing the spread plate technique, where a specified volume of microbial inoculum is evenly distributed over the entire agar surface. This allows for growth in the presence of the extract under examination.

Test Microorganisms and Growth Media

The following clinically isolated microorganisms Gram positive *Staphylococcus aureus*, Gram negative *E. coli* was chosen based on their clinical and pharmacological importance. The bacterial strains were grown in the nutrient broth media (Himedia Labs Pvt.Ltd, Mumbai) at 37°C and maintained on nutrient agar slants (Himedia Labs Pvt.Ltd, Mumbai) at 4°C. The stock cultures were maintained at 4°C.

Mueller Hinton Agar (MHA) medium was used for determination of susceptibility of microorganisms to antimicrobial agents. Suspended 38 grams powder mixture (MHA) in 1000 ml distilled water. Heated until it boils to dissolve the medium completely and checked the pH (7.2). Sterilized the prepared media by autoclaving at 15 lbs pressure (121°C) for 15 minutes and cooled to 45-50°C. Mixed well and poured into sterile petri plates.

Antimicrobial activity testing was carried out by using agar cup method

For the determination of zone of inhibition, Silk sericin prepared (F1, F2 & F3) were taken as the sample, pure Amoxicillin (Himedia Labs Pvt.Ltd, Mumbai) dissolved (1mg/ml) in dimethyl sulfoxide (DMSO) -Nice chemicals Pvt. Ltd, Kochi) was taken (at a conc. of 20 µg/ml) as a standard antibiotic for comparison of the results. Products (F1, F2 & F3) were screened for antibacterial activity against *E. coli* (Gram- negative) and *Staphylococcus aureus* (Gram- positive) bacteria.

MH agar plates were inoculated with a predetermined quantity of the test microorganism using the spread plate method with a sterile glass spreader, and then allowed to stay. A sterile cork borer with a diameter of 6 mm was used to create four holes in the Petri dishes that contained the bacterial culture. The Products (100 µl of F1, F2 & F3) were added into the 6mm diameter wells made in inoculated Petri dishes. Amoxicillin (20µg/mL) was used as the standard. Studies were performed in triplicates and the mean value was calculated. The cultures were kept for 4 hours at 2–8°C for the antimicrobial metabolite diffusion and thereafter they were incubated for 18 hrs at 37 °C for the growth of test micro-organisms. The sensitivities of the microorganism species to the extract were determined by measuring the sizes of inhibitory zones (including the diameter of agar cup) on the agar surface around the wells. The zone of inhibition was measured in mm using a zone scale and values tabulated.

10. Stability Studies

Stability studies were performed on all three formulations (F1, F2, F3) by storing them at room temperature. These formulations were evaluated over a month, with assessments conducted on the 0th, 10th, 20th, and 30th days for parameters such as pH, appearance, homogeneity, and consistency.

RESULTS AND DISCUSSIONS

1. Physical Evaluation

Sl. No	Formulation	Colour	Odour	Texture
1.	F1	White	Characteristic Odour	Smooth
2.	F2	White	Characteristic Odour	Smooth
3.	F3	white	Characteristic Odour	Smooth

2. Homogeneity

Formulation	F1	F2	F3
Homogeneity	Good	Very Good	Good

The analysis of the formulation's homogeneity indicated a uniform composition and no particles detected.

3. Skin Irritation Test

All the formulation were subjected to irritation testing to ensure they are safe for skin use. The formulation did not exhibit any signs of irritation, redness, or itching throughout the study.

Formulation	F1	F2	F3
Irritation	Absent	Absent	Absent

4. pH Value

The pH of the face serum ranges from 4-6. Deviations from this range, whether too high or too low, can lead to skin irritation.

Formulation	F1	F2	F3
pH	5.67	5.43	5.89

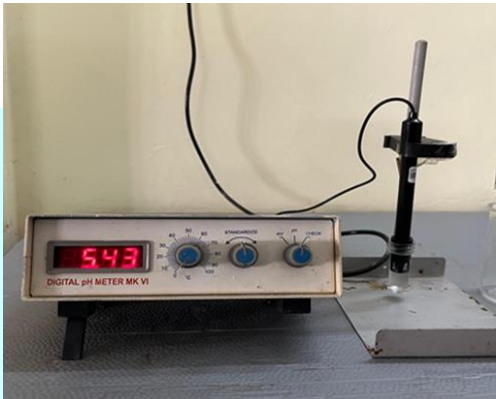


Figure 23: pH testing by using pH meter

5. Spreadability

The spreadability test evaluates the ease with which a face serum can be applied to the skin.

Formulation	F1	F2	F3
Spreadability g.cm/s	1,282	1,388	1,515.15



Figure 24: Testing spreadability by using spreadability apparatus

6. Cyclic temperature

Parameter	Storage	Stability
Cold temperature	Amber bottle	Stable
	Transparent bottle	Stable
Room temperature	Amber bottle	Stable
	Transparent bottle	Unstable



Figure 25: Formulation that affected oxidation

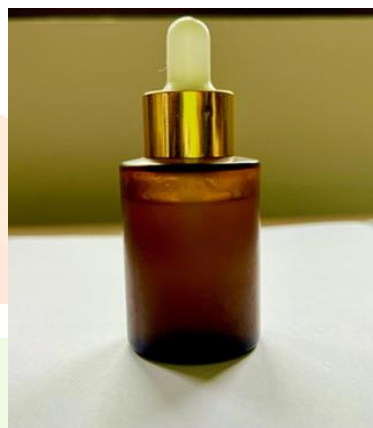


Figure 26: Face Serum stored in amber coloured bottle

7. Anti-oxidant test (FRAP Assay)

FRAP test was conducted to evaluate the antioxidant properties of the formulation. The presence of a blue color was noted, signifying the conversion of ferric ions to ferrous ions. This blue colour in the formulation serves as an indicator of its antioxidant property.



Figure 27: FRAP test

8. Viscosity

The viscosity was assessed using a Brookfield viscometer set at a speed of 100 rpm, utilizing spindle type S63. The measured viscosity was found to be 16.8 cP.



Figure 28: Testing viscosity using Brookfield apparatus

9. Antimicrobial Assay

Microorganism	Zone of inhibition in mm.			
	STD Amoxicillin (20µg/mL)	TEST F1	TEST F2	TEST F3
<i>E. coli</i>	24	-	12	-
<i>S. aureus</i>	30	-	-	-

The sample F2 and the standard (Amoxicillin at a concentration of 20µg/mL) inhibited the growth of *E. coli* species tested. The intensity of the inhibition for F2 was recorded to be as an average of 12mm against the average zone value of 24mm for Standard. Rather than the standard (30 mm) *S. aureus* didn't show sensitivity against the formulated products. From the measurements we obtained, it can be observed that F2 was effective against Gram negative Bacteria.



Figure 29: Antimicrobial assay by using *E. coli* (gram negative)



Figure 30: Antimicrobial assay by using *staphylococcus aureus* (gram positive)

10. Stability Studies

Days	Formulation	pH	Appearance	Homogeneity	Consistency
0	F1	5.6	NC	**	**
	F2	5.4	NC	**	**
	F3	5.8	NC	**	**
10	F1	5.5	NC	**	*
	F2	5.4	NC	**	**
	F3	5.8	NC	**	*
20	F1	5.5	NC	*	*
	F2	5.43	NC	**	**
	F3	5.7	NC	*	W
30	F1	5.6	NC	*	W
	F2	5.4	NC	**	**
	F3	5.7	NC	*	W

NC - No change

** - Good

*- Satisfactory

W - Watery

CONCLUSION

In this project, we explore a face serum suitable for all skin types. This serum offers several advantages, including the reduction of pigmentation due to its antityrosinase properties and its ability to moisturize the skin. The presence of sericin provides antioxidant benefits, aiding in the reduction of reactive oxygen species. This versatile serum can be applied both during the day and at night. With the inclusion of hyaluronic acid, it ensures the skin remains hydrated and supple. Additionally, the combination of sericin, hyaluronic acid, and vitamin E contributes to minimizing the appearance of wrinkles. Furthermore, this face serum is effective in alleviating acne scars, swelling, and redness. Currently, due to the extensive variety of cosmetic products available in the market, consumers are placing significant emphasis on choosing cosmetics to create a high-quality formula for new products. Certain ingredients target specific skin concerns; therefore, a blend of the highest quality components may yield remarkable benefits. Many cosmetics on the market include harmful substances such as PFAS (poly fluoroalkylated substances) and phthalates. PFAS is often referred to as a "forever chemical" due to its persistence in the environment. Our emphasis has been on developing products that exclude these ingredients. It is important to recognize that personal experiences can differ significantly, making it crucial to select products that align with your skin type and particular requirements. Always conduct a patch test with new products and seek advice from a dermatologist if you have any concerns regarding possible allergens or sensitivities. The conclusion drawn is that a facial serum is a valuable asset to the cosmetic industry, making its incorporation essential in any skincare routine.

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