IJBPAS, June, 2026, 15(6): 2727-2746

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

POLYHERBAL MULTIPURPOSE FACE SERUM: FORMULATION AND EVALUATION

ASTHANA SHILAKARI G*, SAI YASVITHA R., G. VISWAS CHOWDARY AND T N G ABHIRAM

Department of Pharmaceutics, Gokaraju Rangaraju College of Pharmacy, Hyderabad, India

***Corresponding Author: Dr. Gyati Shilakari Asthana: E Mail: gyatimmu@gmail.com**

Received 8th June 2025; Revised 6th July 2025; Accepted 9th Sept. 2025; Available online 1st June 2026

https://doi.org/10.31032/IJBPAS/2026/15.6.10247

ABSTRACT

The creation and assessment of a multipurpose polyherbal face serum enhanced with natural extracts such as fenugreek, cucumber, rice, guava leaves, and aloe vera are the main objectives of the present study. The formulation sought to preserve stability and safety for cosmetic use while offering antibacterial, antioxidant, and skin-nourishing properties. Numerous criteria, including pH, viscosity, spreadability, stability, and biological activity, were evaluated for the produced formulations. Among the developed formulations, F3 exhibited superior performance. in the test, exhibiting excellent antibacterial activity against both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli*) bacteria, appropriate pH (4.1–6.7), and improved spreadability (7.3 g.cm/s). Additionally, with a zone of inhibition similar to that of common antifungal drugs, the serum demonstrated exceptional antifungal activities, especially against *Aspergillus niger*. F3 had the highest hydrogen peroxide scavenging ability (85%), according to an antioxidant activity study, which suggests that it could be a good option for lowering oxidative stress on the skin. The serum's endurance was guaranteed by stability tests conducted over a month, which showed no phase separation or physical alterations. Its safety for topical use was confirmed by skin irritation tests that revealed no erythema, oedema, or negative reactions. Excellent stability, antimicrobial, and antioxidant qualities were demonstrated by the developed herbal face serum formulation, especially F3, while preserving a pH that was suitable for the skin. These results imply that the created serum is both safe and effective for use in skincare products.

Keywords: Herbal face serum, Antioxidant activity, antimicrobial properties, fenugreek seeds, guava leaves, rice water

INTRODUCTION:

Herbal extracts, as the name suggests, are prepared from herbs that are mentioned historically in the Bible, Vedas, and Unani writings. The term "herbal cosmetics" refers to products manufactured with a range of permitted cosmetic ingredients **[1]**.

Face serum is a very concentrated blend of water or oil, much like any other cream. Serums, also known as concentrates, can address cosmetic issues more quickly and efficiently than creams since they contain approximately ten times as many physiologically active ingredients. Face serums contain a variety of active ingredients that aid in the treatment of various skin disorders. In addition to treating a specific skin ailment, they contain a blend of active compounds that address other associated problems. Serums are lighter skincare formulas than moisturizers **[2]**. Serum contains a variety of healthy active substances, including ceramides, amino acids, and antioxidants. This explains why face serum is always the priciest item in a skincare package. Antioxidants, cell-communicating ingredients, and skin-identical compounds should be present in all products, including moisturizers, anti-aging or anti-wrinkle treatments, and skin serums. Even though the skin is the body's largest and most protective organ, dry patches can occasionally appear due to several factors, including pollution, UV radiation exposure, and makeup that can irritate or induce allergic reactions if left on all night **[3]**. Various types of herbal serums are explained in the following section, **Figure 1A**.

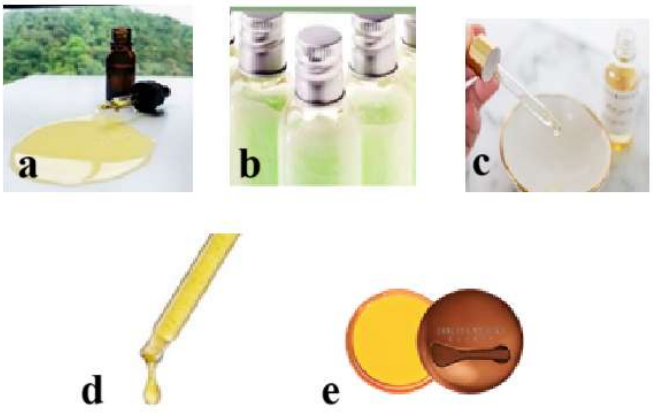


Figure 1A: Types Of Face Serums (a) Oil serum (b) Gel serum (c) Water-based serum (d) Emulsion serum (e) Pressed balm serum (f) Soxhlet apparatus

1. Oil serum

Out of all the face serums, the oil serum is the simplest to produce. Usually, it starts with a foundation of premium, quickly absorbing carrier oils, sometimes referred to as "dry" oils. Apart from their ability to

hydrate and repair the skin's barrier, these premium oils include polyphenols, important fatty acids, and other substances the skin may absorb.

2. Gel serum

Gel serums create the appearance of temporary lifting or firming in specific facial areas by giving the skin a "tightening" sensation. The formulation's water-based nature provides a chance to add hydrophilic plant extracts or attract water, which may be good for the skin.

3. Water-based serum

Although they could contain very little or no gums and thickeners, water-based serums are similar to gel serums. A water-based face serum would be used to apply high-performance hydrophilic plant extracts that are trapped against the skin underneath a cream or lotion. To encourage greater penetration of water-based compounds into the skin and move their high-performance components a little deeper into the layers of the skin, it is best to layer an anti-aging face mist under an emulsion and then an oil. An occlusive barrier created by the oils will encourage greater component penetration.

4. Emulsion serum

An emulsion-based face serum is a moisturizing product with strong active ingredients that strengthen the skin's barrier function. Two immiscible phases are combined to form an emulsion, water, and oil, naturally resistant to mixing. An emulsifier is used to bond the water and oil together, keeping them stable. An emulsion deeply delivers the active ingredients into the skin's tissues. An oil and water blend is the best way to get beyond the skin's barrier, which prevents many cosmetic components from penetrating the dermis. The emulsion's moisturizing qualities also contribute to the skin's barrier strengthening.

5. Pressed balm serum:

A balm serum contains active ingredients that are oil-soluble (lipophilic) and may be good for the skin in addition to the traditional balm base of butter, waxes, and oils. The occlusive barrier that the butter and waxes create on the skin nourishes and moisturizes it while enabling the active ingredients in the pressed serum to perform their functions. Thousands of beautiful plant oils can be mixed with dozens of fascinating, unusual butter and waxes to create a balm serum **[4]**.

Various advantages and disadvantages of face serum

Face serum provides many benefits, such as calming irritated skin, absorbing fast, improving the look of fine lines and wrinkles, preventing free radical damage to the face and further damage, perhaps producing more noticeable results, feeling light on the skin, and enhancing skin elasticity and texture. Skin is nourished and hydrated, and hyperpigmentation is lessened.

However, serum offers expensive advantages, are associated with some of disadvantages may penetrate too quickly, be irritating, not appropriate for people with long-term skin disorders like rosacea or eczema, have potent ingredients that could cause irritation or allergic reactions, need proper storage and packaging are essential to maintain the effectiveness, causes adverse effects by using multiple products **[5, 6]**.

HERBAL SERUM:

Herbal serum is a concentrated skincare product made primarily from natural plant-based ingredients, such as herbal extracts, essential oils, and bioactive compounds derived from medicinal plants. Herbal serums are safer and more skin-friendly than chemical serums, as they are made from natural plant extracts with minimal side effects. All skin types can benefit from their anti-inflammatory, antioxidant, and regenerative properties, especially sensitive skin. Unlike chemical serums, which may irritate, herbal serums promote long-term skin health and are also eco-friendly. Natural elements such as fenugreek, rice, cucumber, rice extract, coconut oil, pomegranate peel powder, orange peel powder, rose water, and aloe vera are included in this herbal face serum formulation **[7]**. Herbal serums are often made from a variety of common plants, such as pomegranate, cucumber, fenugreek, rice, guava, aloe vera, and orange. The section that follows goes into detail about a few of these plants.

VARIOUS COMMON HERBS USED IN HERBAL SERUM PREPARATION (Table 1 supplementary data):

Cucumber: Active Ingredient: Cucumber peel extract, Biological Name: *Cucumis sativus*

Family: Cucurbitaceae, Use: Extract from cucumber peels helps the skin from UV radiation and environmental pollution, combating free radicals that contribute to wrinkles and signs of aging **[8]**.

Fenugreek: Since ancient times, fenugreek, often referred to as methi, has been utilised as a treatment for several illnesses. Folic acid, thiamine, vitamins A, C, K, B6, riboflavin, niacin, iron, magnesium, potassium, zinc, selenium, calcium, copper, and more are among its many healthy ingredients. This nutrient-dense superfood gives the skin vital nourishment, improves natural skin brightness, and fights free radical damage. It also aids in bringing the skin's pH levels into balance. Fenugreek is very good for skin health because of its inherent anti-inflammatory, antibacterial, and antioxidant qualities. In addition to creating radiant skin, it may be used for cleansing, anti-aging, moisturizing, and minimizing acne, dark circles, and blemishes **[9]**.

Rice: Rice is a natural skincare cure that contains vitamins, minerals, and amino

acids that are believed to preserve and restore skin. Rice has anti-aging properties, brightens the complexion, strengthens the skin barrier, and can lessen facial oiliness **[10]**.

Guava: Bioactive compounds and other health-promoting micro- and macronutrients can be found in abundance in guava leaves. Their total phenolic components include 103 mg of ascorbic acid, 1717 mg of gallic acid equivalents (GAE), and 82.47% moisture **[11]**.

Aloe vera: Fibroblasts, which produce collagen and elastin fibers, are stimulated by aloe vera, increasing the skin's suppleness and decreasing wrinkles. It has long been used to treat wounds and infections because of its antibacterial and antioxidant qualities. Because aloe vera blocks UVA and UVB radiation, it also helps to preserve the natural moisture balance of the skin. It also helps avoid sunburn **[4]**.

Orange Peel Powder: In addition to important elements like Ca, Mg, and vitamins A, B, and C, orange peels are a great source of flavonoids, phytochemicals, and antioxidants. They help protect the skin from UV rays **[12]**.

Pomegranate Peel powder: Pomegranate peel contains vitamin C, which keeps the skin moisturized, encourages skin cell renewal, and helps stop moisture loss. Furthermore, it reduces pore size, tightens the skin, and reduces the appearance of wrinkles and fine lines **[7]**.

The present research aims to formulate a multipurpose polyherbal face serum composed of various extractions such as rice, cucumber, guava leaves, fenugreek, aloe vera, orange peel, and pomegranate peel powder for antioxidant and antimicrobial, antiaging, and hydration purposes. Numerous factors, including pH, appearance, viscosity, spreadability, irritancy, antibacterial activity, antioxidant activity, and stability tests, were assessed for the serum.

SUBJECTS AND MATERIALS:

MATERIALS: Various materials used to conduct the present work are displayed in **Table 1**.

Table 1: Components of the polyherbal face serum formulation

S. No.	INGREDIENTS	BIOLOGICAL NAME	ROLE OF INGREDIENTS
1	Fenugreek	*Trigonella foenum-graecum*	bright skin, cleanser, anti-aging, moisturising, and minimising acne, dark circles, and imperfections.
2	Cucumber	*Cucumis sativus*	Protect against UV rays, antioxidant
3	Rice	*Oryza sativa*	anti-ageing, brightens skin, Reduce oiliness
4	Guava	*Psidium guajava*	Helps To Treat Wrinkles, Lighten Dark Spots, Treats Acne and Blemishes, Remove Blackhead, Relieves Itching on Face
5	Aloe vera	*Aloe barbadensis miller*	Anti-aging
6	Orange Peel powder	*Citrus sinensis*	Antioxidants, protect the skin from UV rays.
7	Pomegranate Peel powder	*Punica granatum*	Anti-aging, boosts skin cell regeneration, hydrating

8	**Glycerin**	**-**	**Preservative, Anti-ageing, de-tanning agent, gives skin a glowy look.**
9	**Vitamin E**	**-**	**Antioxidant**
10	**Lemon oil**	***Citrus limon***	**Antioxidants, antibacterial, antiviral, antifungal, astringent, beneficial for treating a variety of microbiological skin conditions and for cleansing small cuts, abrasions, and lesions on the skin.**
11	**Coconut oil**	**Cocos nucifera**	**Nourishing, keeping your skin hydrated, protecting, and retaining moisture.**
12	**Sodium benzoate**	**-**	**Preservative**
13	**Tween 20**	**-**	**Solubilising agent**
14	**Span 80**	**-**	**Emulsifier**
15	**Rose water**	**-**	**natural skin tone [7-14]**

METHODS

COLLECTION OF HERBS: The plants utilized were bought from the local market and acquired from Gokaraju Rangaraju College of Pharmacy's botanical garden. then roughly ground, dried in the shade, and utilized for extraction **Figure 1B**.

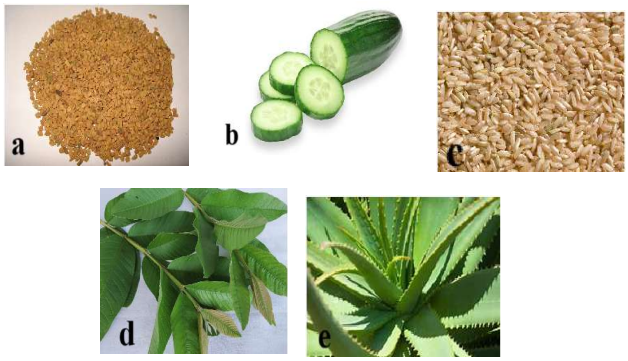


Figure 1B: Crude drugs: (a) Fenugreek, (b) Cucumber, (c) Rice, (d) Guava, (e) Aloe vera

EXTRACTION OF CRUDE DRUGS

a. EXTRACTION OF FENUGREEK: The fenugreek seeds were extracted using the maceration technique. Fenugreek seeds were cleaned, dried, and crushed. The crushed fenugreek seeds are incorporated into distilled water in a 1:10 ratio (Ten parts distilled water to one part seeds) in a sterile container. After swirling, the fluid was allowed to settle to room temperature for 72 hours to make sure the seeds were uniformly distributed throughout the water. During this time, the active components of fenugreek seeds permeate the water and create a fenugreek extract. The extract was kept out of direct sunlight in a sterile, airtight container to prevent degradation of its active components **(Figure 1C a) [9]**.

b. EXTRACTION OF CUCUMBER: Fresh cucumber fruits were taken, thoroughly cleaned, and then rinsed with purified water. After chopping

the cucumber into small pieces, 120 g was added to a 500ml beaker that had been cleaned. To the cucumber combination, add 200ml of deionized water, 1g of methylparaben, and 5ml of glycerine. After that, they were placed in an airtight container, sealed, and stored for five days. At least once a day, give it a little shake. After five days, filtered the extract with a cotton cloth. The cucumber extract was moved to an airtight container and refrigerated until it was needed again **(Figure 1C b) [15]**.

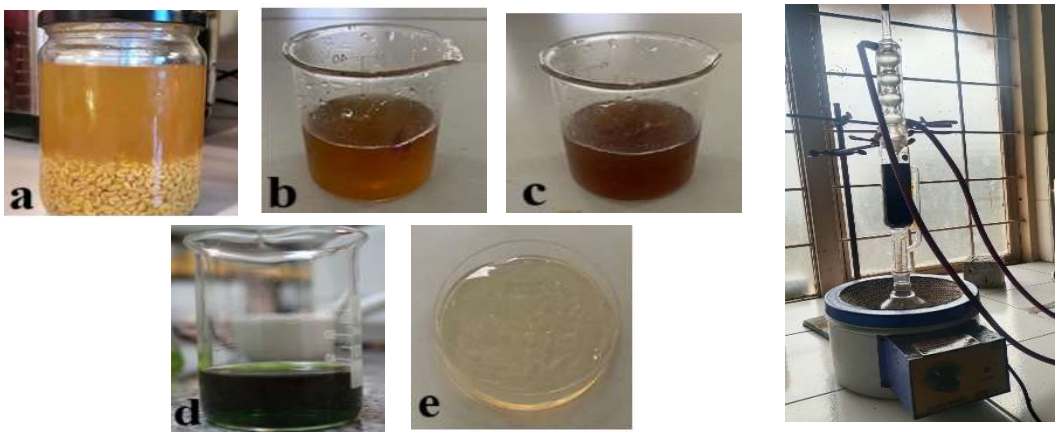


Figure 1C: Extractions of (a) Fenugreek seeds, (b) Cucumber, (c) Brown rice, (d) Guava leaves, (e) Aloe vera

c. **EXTRACTION OF RICE:** Took the brown rice 50g, and kept it in boiling into distilled water. After the rice had cooled completely, the rice was drained to produce the starch suspension. This liquid is referred to as rice water (starch suspension). Rice water includes vitamins, minerals, and amino acids that are proven to preserve and restore skin; it is a natural home remedy **Figure 1C c [10]**.

d. **EXTRACTION OF GUAVA LEAVES:** Leaves were well cleaned, let to dry, then blended into a powder and kept for later use in an airtight container. Vitamin C was extracted from powdered guava leaves using the solvent extraction method **(Figure 1C d)**. After being filled with guava leaf powder, the Soxhlet thimble was placed inside the main chamber and sealed. The chamber containing the solid thimble is flooded with solvent vapour as it ascends to the column. Some part of the non-volatile chemicals dissolve in solvents.

Finally, collect the extract of guava leaf as an organic extract **(Figure 1C d) [11]**.

e. **EXTRACTION OF ALOE VERA:** Aloe vera leaves are collected from the botanical garden of Gokaraju Rangaraju College of Pharmacy. And washed thoroughly with water. Extract the gel from fresh and intact aloe vera leaves. Aloe vera gel was collected **(Figure 1C e)**.

FORMULATION OF MULTIPURPOSE HERBAL FACE SERUM

The polyherbal serum was prepared with the ingredients mentioned in **Table 2**, and the preparation comprises the following

1. Preparation of the water phase
2. Preparation of the oily phase
3. Combination of both phases
4. Addition of powders

Table 2: Composition of Multipurpose Polyherbal Face Serum

AQUEOUS PHASE				OIL PHASE			
INGREDIENTS	**F1**	**F2**	**F3**	**INGREDIENTS**	**F1**	**F2**	**F3**
Fenugreek extract	**2ml**	**2.5ml**	**3ml**	**Coconut Oil**	**2ml**	**2.5ml**	**3ml**
Cucumber extract	**1ml**	**1.5ml**	**2ml**	**Lemon Oil**	**1ml**	**1ml**	**1ml**
Rice extract	**1ml**	**1.5ml**	**2ml**	**Vitamin E**	**1.5ml**	**2ml**	**2.5ml**
Guava leaf extract	**2ml**	**2.5ml**	**3ml**	**Tween 20**	**2%**	**2%**	**2%**
Aloe vera gel	**2ml**	**2ml**	**2ml**	**-**	**-**	**-**	**-**
Glycerine	**2ml**	**2ml**	**2ml**	**-**	**-**	**-**	**-**
Rose water	**qs**	**Qs**	**qs**	**Pomegranate peel powder**	**1g**	**2g**	**3g**
Sodium benzoate	**0.1g**	**0.1g**	**0.1g**	**Orange peel powder**	**1g**	**2g**	**3g**

STEP 1: Water phase: It consists of aqueous extracts of fenugreek, cucumber, rice, guava, aloe vera.

- In sanitized beaker combine all the water-based extracts fenugreek, cucumber, rice, guava, aloe vera as mentioned in **Table 2**.
- Glycerine: Glycerine, a humectant, draws moisture into the skin from the surroundings to keep it hydrated and stop it from drying out. Its moisturising qualities make it a popular ingredient in skincare formulas.
- Water (qs): This is the vehicle that allows all the active ingredients to be dispersed and applied easily to the skin. The amount added is "quantum sufficient" (qs), meaning just enough to make up the desired volume of the water phase.
- Sodium Benzoate: This is a preservative that prevents microbial growth in water-based products. By preventing bacteria, fungi, and other microbes from growing it contributes to the end product's safety and shelf life.

The water phase was mixed thoroughly to ensure all ingredients are well combined and evenly distributed before moving to the next phase.

STEP 2: Oil Phase: It consists of coconut oil, lemon oil, vitamin E, and tween 20

The water and oil phases can mix to form a stable emulsion thanks to the oils and emulsifiers in the oil phase, as mentioned in **Table 2**.

Coconut oil: Rich in vitamins and fatty acids, this oil is lightweight and non-comedogenic. It helps retain moisture and contains antioxidant qualities, which leave the skin feeling nourished and silky.

Lemon oil: Well-known for its invigorating citrus aroma, lemon oil also contains a wealth of antioxidants and natural antibacterial qualities that assist in brightening and toning the skin.

Vitamin E: A potent antioxidant that promotes the skin's natural barrier function and shields the skin from environmental stresses. It also has anti-inflammatory and moisturizing properties.

Tween 20: serves as an emulsifier to create a stable emulsion. Additionally, it functions as a non-ionic surfactant. When combined, these emulsifiers facilitate the blending of the two phases, ensuring the product remains uniform over time.

STEP 3: Preparation of emulsion phase

Once both the water and oil phases were prepared, with constant stirring, the oil phase was gradually added to the water phase. This is an important step for creating a smooth, stable emulsion. If you have access to a handheld mixer or an immersion blender, this can help emulsify the mixture more efficiently, resulting in a more uniform texture.

STEP 4: Adding Powders

The final step involves adding powdered ingredients as mentioned in **Table 2** to the emulsion once it is stable.

Orange peel powder: Rich in vitamin C, this powder has lightening and exfoliating qualities. It aids in the elimination of dead skin cells, resulting in a smoother, more radiant complexion.

Pomegranate powder: Pomegranate powder, which is rich in antioxidants, can encourage skin renewal and protect the skin from the elements. It is also well-known for its anti-aging qualities. These powders should be added after the emulsion has stabilized to prevent them from clumping or interfering with the emulsion process. Stirring thoroughly ensures that the powders are evenly distributed throughout the mixture.

EVALUATION OF HERBAL SERUM:

The various herbal serums are evaluated for organoleptic properties, irritancy, pH, viscosity, spreadability, antimicrobial, and antioxidant activity.

Organoleptic properties:

Physical Appearance: Color, Odour, and texture: The appearance of the serum, including its color, fragrance, and viscosity, should be visually appealing and consistent with what is expected of an anti-aging

product. Any change in these properties over time (such as discoloration or separation) could indicate instability. Color, Odour, and texture were determined visually, and the results were noted **[7]**.

Irritancy: Mark the 1 cm2 region on the left-hand dorsal surface to indicate irritation. After that, the area was treated with the serum, and the time was noted. The region was checked for any irritating effects, erythema, or oedema after a predetermined amount of time, and if any were found, they were reported **[7]**.

pH Determination: The pH level is critical for the stability and effectiveness of the serum. Skin-friendly formulations typically have a pH range of 4.1-6.7. This ensures that the product won't irritate the skin and supports the skin's natural barrier. pH can be determined by a pH meter **Figure 1D a [2]**.

Figure 1D: Determination of evaluation parameters of herbal serum (a) pH meter, (b) Brookfield viscometer, (c) Spreadability

Viscosity: The formulation's viscosity was assessed using a Brookfield viscometer with a spindle-type model S64 at 100 rpm and 25 °C **Figure 1Db**. Readings were taken after the spindle was submerged in 5 ml of the serum for around 5 minutes **[2]**.

Spreadability: The time taken for two slides separated by a layer of serum to separate from the serum under a particular load was used to calculate the spreadability. Two sets of standard-sized glass slides were taken. After that, the serum formulation was put onto a slide that had been accurately measured. A second slide was then placed over the formulation. The serum between the two slides was then uniformly compressed into a thin layer by applying a weight on the upper slide, as shown in **Figure 1Dc**. After the weight was taken off, the extra formulation that had stuck to the slides was scraped off. Because of the weight attached to it, the upper slide was able to move freely. It was noted how long it took for the top slide to come off **[16]**.

Spreadability = m × l/T,

where l is the length (5 cm), t is the duration (in sec), and m is the standard weight (50g).

Dye Test: Using Sudan III dye, a dye test was carried out and viewed under a microscope. A little slide was covered with

a cover slip, and Sudan III was used to dilute a drop of the serum.

dye. It indicates that the emulsion is of the o/w type if the dispersed globules appear crimson. It is w/o type if the continuous phase appears red and the continuous phase is colourless.

Antimicrobial activity:

Anti-bacterial activity: The antibacterial activity of different serums against both Gram-positive and Gram-negative bacteria was determined using the traditional agar well diffusion method. An autoclave was used to prepare and sterilise the nutrient agar medium for 20 minutes at 121 °C and 15 pounds of pressure. Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli*) bacteria were injected into a sterilised molten agar medium via aseptic transfer beneath a laminar air flow bench. The agar medium was immediately transferred onto sterile petri dishes and left to solidify. On Petri plates, wells were made in the proper places using a sterile borer (8mm). After adding the serum to the wells, the Petri plates were left aside to allow the sample to diffuse through the agar medium. The plates were then incubated for 24 hrs at 37 °C. A ruler was used to measure the zones surrounding each well that showed an inhibition zone around the cup or well.

Antifungal activity: The antifungal activity of the proposed formulations was done using Sabouraud agar media. An autoclave was used to manufacture and sterilise Sabouraud agar media for 20 minutes at 121 °C and 15 lbs of pressure. By aseptic transfer beneath the laminar air flow bench, sterilised molten subouraud agar medium was inoculated with Gram-positive fungi (*Candida albicans*) and Gram-negative fungi (*Aspergillus niger*) independently. The sterile Petri plates were immediately filled with the inoculated Subouraud agar medium, which was then left to solidify. On Petri plates, wells were made in the proper places using a sterile borer (6mm). After adding the serum to the wells, the Petri plates were left aside to allow the sample to diffuse through the agar medium. After that, the plates were incubated at 25–28 °C for 24 hours. A ruler was used to measure the zones surrounding each well that showed an inhibition zone around the cup or well **[16]**.

Antioxidant Activity: It was determined by H_2O_2 Free Radical Scavenging activity assay **[17]**.

Method: The plant extract's capacity to scavenge hydrogen peroxide, a dangerous reactive oxygen species, is assessed by the H_2O_2 assay. Cells and tissues may sustain oxidative damage as a result of hydrogen peroxide. The assay gauges the plant extract's antioxidant capacity by measuring the drop in hydrogen peroxide concentration following exposure.

Principle: The scavenging ability of natural antioxidants found in plant extracts against

hydrogen peroxide (H_2O_2) has been thoroughly studied by measuring the decrease of H_2O_2 in an incubation system containing H_2O_2 and the scavenger using the conventional UV technique at 230 nm.

Preparation of reagents: PBS (pH 7.4): 1000 mL of water was taken to dissolve 6.8 gm of K_2HPO_4, and 1.5 g of NaOH.

1. Hydrogen peroxide (40 mM) preparation: 0.228 ml of H_2O_2, were diluted in 50 ml phosphate buffer.
2. Ascorbic acid (standard) Preparation: 10 mg of ascorbic acid was diluted in 10 ml of phosphate buffer.
3. Sample preparation involves diluting 10 mg of the sample in 10 ml of PBS (pH 7.4).

Method

- A 40mM of H_2O_2 was mixed in PBS (pH 7.4)
- In distilled water, sample were added to H_2O_2 (0.6ml, 40mM)
- Ascorbic acid (100 µl) was added to each test tube.
- Phosphate buffer (pH 7.4) was then added to each test tube until the volume reached 4 ml.
- The standard consisted of the same Ascorbic acid series (phosphate buffer, H_2O_2, and Ascorbic acid) minus the sample.
- Every test tube was allowed to sit at room temperature for 10 minutes.
- The H_2O_2 absorbance was measured at 230 nm compared to the blank.
- H_2O_2 scavenging activity was performed for the marketed herbal serum for comparison with the developed formulations.
- H_2O_2 scavenging activity percentage computed using the formula below in comparison to the standard

$$\text{Scavenging Activity (\%)} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Stability Studies: Stability tests were performed on the chosen final formulation. It was done to ascertain the product's safety by assessing its chemical and physical stability. The stability investigations were conducted following accepted practices. A short-term accelerated stability investigation lasting one month was carried out for the produced formulation. Several temperature ranges, including 3-5°C and 40°C±2%, were used to store the samples **[1]**.

RESULTS:

Physical evaluation: All of the produced formulations underwent a visual physical examination. All the serums are green in color, aromatic in odour, and smooth in texture, as shown in **Figure 2 and Table 3**.

Irritation test: By applying serum to the skin's most sensitive area, an irritability test was conducted. After a specific duration, the site was examined for any irritant effects,

erythema, or edema. Following the application of any formulation, erythema and oedema were not seen, nor was there any irritation as displayed in **Table 4**, supplementary data. This indicates that all the herbal serum formulations are skin-compatible and safe.

pH Test: Each created composition's pH was measured using a pH meter, and it was found within the range of 4.1 – 6.7. All of the formulations had good pH values, and it was within the skin pH range as shown in **Table 5**.

Viscosity: Table 5 displays the results of utilizing Brookfield to determine the formulation's viscosity. Serum has a general viscosity for application, as evidenced by the created formulations' viscosity, which was determined to be between 915 and 391 cps.

Type of Emulsion Test:

Dye Test: After diluting a small amount of serum with Sudan III, it was put on a glass plate and examined under a microscope. The dispersed globules were discovered to appear red and the continuous phase colourless, indicating that the serum is an emulsion of the O/W **Figure 3**.

Spreadability: The formulations' spreadability was tested, and the findings are displayed in **Table 5**. The range of spreadability was determined to be between 6.1 and 7.3 g.cm/s. It has good spreadability and takes less time for the two slides to separate.

The F3 formulation was deemed the ultimate one since it demonstrated good spreadability and required less time to separate two slides than the others.

Antimicrobial activity: The generated herbal serum was tested for antimicrobial efficacy against bacteria and fungi **[16]**.

Using the agar well diffusion method, antibacterial activity was assessed using Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli*) bacteria as well as fungal strains *Candida albicans* and *Aspergillus niger*. The zone of inhibition was measured, as indicated in **Figure 5**, and compared with the commercial formulation, as indicated in **Table 6**. Comparing F3 to other formulations like F1 and F2, it was discovered that F3 was exhibiting a good zone of inhibition.

Comparing the F3 formulation to the F1 and F2 formulations, the F3 formulation showed the largest zone of inhibition against bacteria and fungi of all the herbal serums. **Table 6 and Figure 5(i)** demonstrate that the zone of inhibition was greater for *E. coli* than for *S. aureus*.

Likewise, a greater zone of inhibition was noted in the instance of *Aspergillus niger* (Gram-negative) as compared to *Candida albicans* (Gram-positive) as shown in **Table 6 and Figure 5 (ii)**. The zone of inhibition of the formulations is compared with the

marketed formulation, **Figure 5(iii), Table 6**.

Antioxidant Activity:

It was determined using the H_2O_2 assay method, and the results are displayed in **Figure 6**. All the formulations show antioxidant activity. Among all, the F3 formulation shows the highest, i.e., 85% antioxidant activity compared to other serums. F1 displays only 12.9% antioxidant activity. The antioxidant activity of the marketed formulation was recorded as 92.7%. The F3 formulation displayed 85% antioxidant activity, which is near the marketed formulation, as shown in **Figure 6 and Table 7**.

Stability studies:

Stability tests were conducted at various storage temperatures, including 3-5°C and 40°C±2%, and appearance and phase separation were recorded. There was no separation and no change in the formulation. Thus, as indicated in **Table 8** all of the herbal serums are determined to remain stable after the storage period.

Statistical analysis used:

Comparative evaluation was done by measuring zones of inhibition (antimicrobial activity), pH, viscosity, spreadability, and hydrogen peroxide scavenging percentage (antioxidant assay). No complex statistical modeling was mentioned; results were reported as mean values with standard deviations where applicable.

Figure 2: Various Polyherbal face serums

Table 3: Polyherbal face serum

S. No.	Physical Parameters	F1	F2	F3
1	Colour	Dark green	Dark green	Dark green
2	Odour	Aromatic	Aromatic	Aromatic
3	Texture	Smooth	Smooth	Smooth

Table 4: Result of Irritant test

S.no	Formulation Codes	Irritant effect	Erythema	Edema
1	F1	Nil	Nil	Nil
2	F2	Nil	Nil	Nil
3	F3	Nil	Nil	Nil

Table 5: pH, Viscosity and Spreadability of herbal serum

S. No.	Formulation codes	pH	Viscosity (cps)	Time (sec)	Spreadability (g.cm/sec)
1	F1	5.3	915	15	5.2
2	F2	6.1	568	12	6.1
3	F3	6.6	391	9	7.3

Figure 3: Dye test of Serum under a microscope

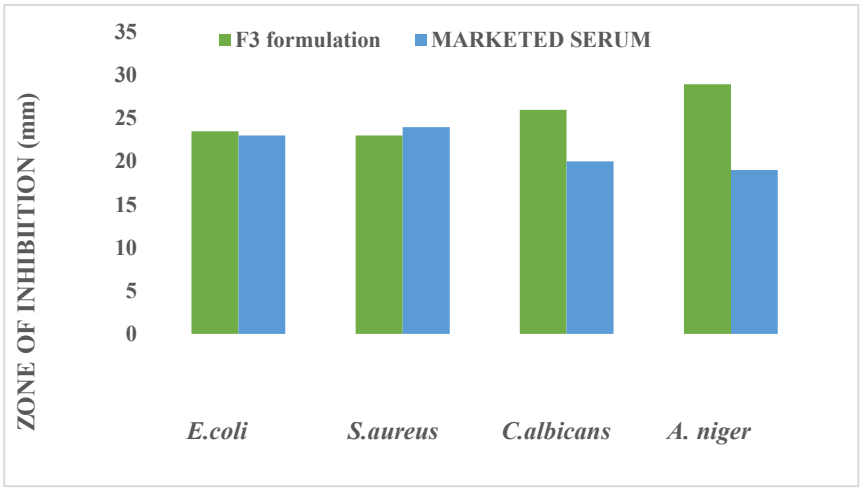
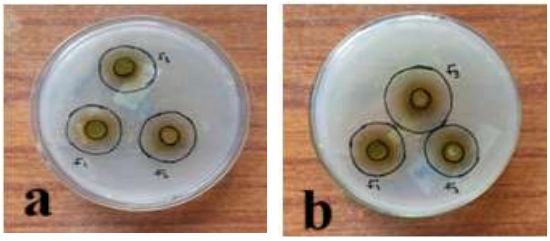


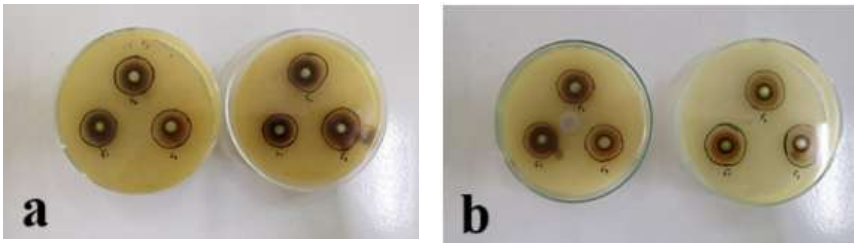
Figure 4: Comparison of antimicrobial activity of F3 formulation to marketed serum

Table 6: Herbal serum's antibacterial and antifungal properties (Zone of inhibition) mm

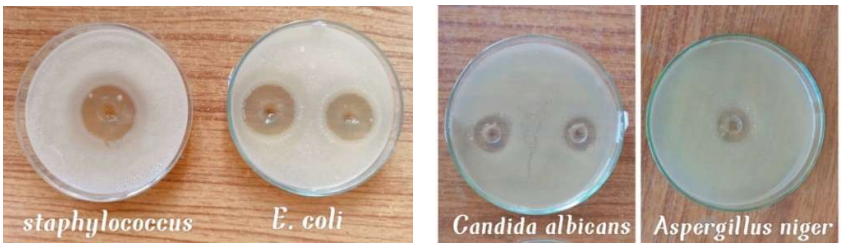
Microorganisms	Herbal serum formulations zone of inhibition (mm)			
	F1	F2	F3	Marketed herbal serum
E. coli	22.0±0.02	23.0±0.03	23.5±0.02	23±0.02
Staphylococcus aureus	21.5±0.02	22.0±0.03	23.0±0.01	24±0.01
Candida albicans	24.0±0.03	25±0.04	26±0.03	20±0.03
Aspergillus niger	22±0.01	23±0.02	29±0.03	19±0.02



(i): (a) *E. coli* (b) *Staphylococcus aureus*



(ii): (a) *Candida albicans* (b) *Aspergillus niger*



(iii): Marketed herbal serum

Figure 5: Zone of inhibition:(i) (a) *E. coli* (b) *Staphylococcus aureus* (ii):(a) *Candida albicans* (b) *Aspergillus niger* (iii): Marketed herbal serum

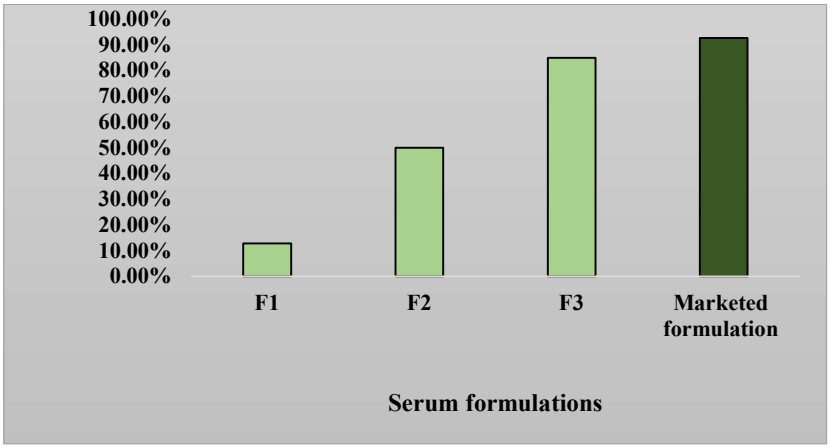


Figure 6: Comparison of the antioxidant activity of serum formulation to marketed serum

Table 7: Antioxidant activity of developed serum

S. No.	Formulation	Absorbance (NM)	% OF H_2O_2
1	Standard	0.486	-
2	F1	0.423	12.9
3	F2	0.243	50
4	F3	0.072	85
5	Marketed formulation	0.035	92.7

Table 8: Stability tests for a month at various temperatures

S. No.	Formulation codes	pH	Appearance	Phase separation
1	F1	5.3	No change	No phase separation
2	F2	6.1	No change	No phase separation
3	F3	6.6	No change	No phase separation

DISCUSSION:

The physical evaluation of the herbal serum formulations (F1, F2, F3) revealed that all formulations had a dark green color due to herbal extract, an aromatic odor due to lemon oil, and a smooth texture. These characteristics indicate that the formulations maintained a consistent appearance and texture, suggesting stability during preparation.

The irritation test results were favourable, as none of the formulations caused any irritation, erythema, or edema after a specific duration of application. This suggests that the herbal serums are safe for use on sensitive skin, making them suitable for a wide range of consumers.

The pH of all the formulations was found to be within the skin-friendly range of 4.1-6.7. The pH values indicate that these formulations are unlikely to disrupt the skin's natural barrier, further enhancing their potential for safe and effective use. The viscosity of the formulations ranged from 915 cps to 391 cps, indicating a suitable consistency for easy application and smooth spreading.

Spreadability testing showed that F3 had the best spreadability, with the shortest time taken for the two slides to separate (9 seconds) and the highest spreadability value of 7.3 g.cm/s. This makes F3 the most desirable formulation in terms of ease of application and even distribution on the skin.

The antimicrobial activity of the formulations was tested against both Gram-positive (*Staphylococcus aureus)* and Gram-negative *(E. coli)* bacteria, as well as fungal strains (*Candida albicans* and *Aspergillus niger*). F3 demonstrated the highest zone of inhibition for both bacteria and fungi, indicating its superior antimicrobial properties compared to F1 and F2. The zone of inhibition was compared with the marketed herbal serum, suggesting that this formulation may be highly effective against microbial growth.

Antioxidant activity testing revealed that F3 showed the highest antioxidant potential, with 85% inhibition of hydrogen peroxide. This suggests that F3 could offer additional benefits such as protecting the skin from oxidative stress, further enhancing its appeal as a skin care product.

Stability studies at room temperature over one month showed that all formulations remained stable, with no change in appearance or phase separation. This indicates that the herbal serum formulations

have good stability and can be expected to maintain their quality over time when stored under normal conditions.

CONCLUSION:

The study successfully developed and evaluated three polyherbal face serum formulations using various herbal extracts known for their skin-enhancing properties. All formulations exhibited favourable physical characteristics, including dark green color, aromatic odor, and smooth texture. The serums were non-irritant, with no signs of erythema or edema, confirming their compatibility with skin. Serums are found to be o/w type of emulsion.

Physicochemical evaluations showed that all formulations had acceptable pH levels (within the skin-compatible range), suitable viscosity, and good spreadability. Among the three, **Formulation F3** consistently outperformed F1 and F2 across various parameters. It showed exceptional antibacterial action against strains of fungi and bacteria, both gram-positive and gram-negative, with the highest zone of inhibition observed for *Aspergillus niger*. F3 also exhibited the **highest antioxidant activity** (85%) and maintained outstanding stability without phase separation or appearance changes under a range of storage conditions. Based on these findings, **F3 formulation can be considered the most effective and stable multipurpose polyherbal face serum**, offering potent antimicrobial, antioxidant, and skin-friendly properties. This formulation holds promising potential for future cosmetic and therapeutic skin care applications.

The herbal serums that contained fenugreek, cucumber, rice, guava leaf, and vitamin E extracts showed a good composition for potential use in the future.

ACKNOWLEDGEMENT:

The Gokaraju Rangaraju College of Pharmacy provided all the facilities needed to carry out the current study, for which the authors are truly grateful.

REFERENCES:

[1] Joshi S, Joshi H, Joshi P, Kamal S. Formulation and Evaluation of a Polyherbal Anti-Aging Face Serum. Human J. 2024;30(5):678–94. https://ijppr.humanjournals.com/wp-content/uploads/2024/06/48.Sweta-Joshi-Hricha-Joshi-Priyanka-Shaurya-Kamal.pdf

[2] Khanna T, Joshi S. Formulation and evaluation of Anti Acne face serum. 2024. 166-17. https://www.plantsjournal.com/archives/2024/vol12issue3/PartB/12-3-19-520.pdf

[3] Gite AV, Udapurkar P, Sanap AS. Formulation and Development of Face Serum. Int J Creat Res. Thoughts. 2023; 11(6): 2320-2882. https://ijcrt.org/papers/IJCRT23nm06095.pdf

[4] Salunke V, Jagdale A, Pangude P, Kawade RM. Formulation and Evaluation of Herbal Face Serum. Int J Creat Res Thoughts. 2024;12(6):j945–j948. https://ijcrt.org/papers/IJCRT24A6109.pdf

[5] Abhirami V, Kombathethil Ali A, PN A, V D, KU R, Varghese J. Formulation and Evaluation of Polyherbal Face Serum: Research Article. Int J Creat Res Thoughts. 2023;11(7). 294-300. https://ijcrt.org/papers/IJCRT2307152.pdf

[6] Adhikar MS, Chavan VA, Gaikwad NB. Formulation and Evaluation of Face Serum Containing *Carica papaya* and *Aloe vera*. Int J Creat Res Thoughts. 2024;12(6):121–3. https://ijcrt.org/papers/IJCRT2406568.pdf

[7] Shikalgar SA, Momin NS. Formulation and Evaluation of Herbal Face Serum. Int Res J Mod Eng Technol Sci. 2024;6:3322–4. https://www.irjmets.com/uploadedfiles/paper/issue_6_june_2024/59465/final/fin_irjmets1719317858.pdf

[8] Pawar AS, Zore S, Pawar PH, Pawar SS. Formulation and Development of Herbal Face Serum on Anti-Aging Containing Kiwi and Cucumber. Int J Creat Res. Thoughts. 2023;8(11). 261-270. https://ijrti.org/papers/IJRTI2311037.pdf

[9] Yeskar H, Makde P, Tiware SA, Shirbhate TM, Thakre SV, Darne CS, *et al*. Formulation and evaluation of a face serum containing fenugreek extract. Int J Basic Clin Pharmacol. 2023;12(6):799–804. https://doi.org/10.18203/2319-2003.ijbcp20233189

[10] Khandekar SS, Ghodke PI, Udapurkar P. Formulation of Herbal Face Serum from Indian Herbal. Int J Creat Res Thoughts. 2023;11(6):815–6. https://www.ijcrt.org/papers/IJCRT2306092.pdf

[11] Biradar AB, Bokade GD, Shelke RU. Formulation and Evaluation of Herbal Face Serum. Int J Pharm Sci. 2024;2(6):595–601. https://doi.org/10.5281/zenodo.11550812

[12] Palave AD, Bhabad R, Kotkar VT. Formulation and Evaluation of Face Serum. Int J Adv Res Innov Ideas Educ. 2024;10(2):4542–50. https://ijariie.com/AdminUploadPdf/FORMULATION_AND_EVALUATION_OF_FACE_SERUM_ijariie23422.pdf

[13] Gite AV. Formulation and Development of Face Serum. Int J Creat Res Thoughts. 2023;11(6):a833–a845. http://www.ijcrt.org/papers/IJCRT2306095.pdf

[14] Teli, B., Kumar, L. S., Chaithra, L. N., Chethana, B. S., Sushmitha, C., Neelambika, H. N., Panchami, M. V., Ravichandran, K. S., & Kavitha, P. N. (2024). Formulation and evaluation of poly herbal face anti-aging serum. International Research Journal of Modernization in Engineering Technology and Science, 6(9), 2714–2723. https://doi.org/10.56726/irjmets61793

[15] Jadhav SA, Gachande SD, Jadhav DS, Gangurde MS. Formulation and Evaluation of Herbal Face Gel Using Cucumber Fruit Extract. Int J Pharm Sci. 2024;2(6):1–9. https://doi.org/10.5281/zenodo.11409503

[16] Shilakari G, U A, G A, L IR. Antimicrobial activity of cream containing polyherbal extract: development, formulation and evaluation. Int J. Pharm Sci Rev Res. 2024;84-91. https://doi.org/10.47583/ijpsrr.2024.v84i04.012

[17] Gupta M, Dey A, Majumder S, Ghosh S, Barui A. Evaluation of antioxidant activity using H_2O_2, DPPH, ABTS methods and phytochemical tests, TPC & TFC of commonly used medicinal plants of West Bengal. Int J Pharm Sci Rev Res. 2022;123-132. https://doi.org/10.47583/ijpsrr.2022.v72i01.018