

# Formulation and Evaluation of Herbal Anti-Oxidant Face Serum from *Bunchosia Armeniaca*

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## ABSTRACT

Wrinkles, dullness, photo-aging and pigmentation are caused by oxidation reactions. *Bunchosia armeniaca* contains antioxidants which can dispose-off free radicals that will damage the skin. Its fruit extract is incorporated into the face serum. Within this experiment, we have formulated and evaluated an herbal antioxidant face serum comprising the extracts of *Bunchosia armeniaca* in combination with the methanolic extracts of *Clitoria ternatea* and *Psidium guajava*. Seventeen different formulations of the herbal face serum were prepared and tested for its pH, viscosity, spreadability, stability, antioxidant activity, etc. Optimization was done using Design Expert Software (DoE), version 13, which helped us to achieve maximum antioxidant power, enhance stability, safety, and efficacy while also ensuring that the serum possesses desirable physical, chemical and sensory properties. The results of this experiment shows that F8 was identified as the optimal antioxidant serum that exhibited these desirable characteristics by demonstrating that it is a safe, natural formulation that effectively repairs facial photodamage, microbial infections, and inflammations, offering a viable alternative to synthetic face serums. Consequently, it shows substantial potential for future development and commercial success.

**Keywords:** Herbal antioxidant face serum, *Bunchosia armeniaca* extract, Optimization, Design Expert Software (DoE), Box- Behnken Design, in vitro DPPH assay, in vitro Anti-inflammatory assay, in vitro Anti-microbial assay.

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**Conflict of interest:** None

## I. INTRODUCTION

The concept of enhancing one's appearance has been a significant part of human culture throughout history. Rather than a modern invention, the pursuit and practice of beautification are timeless, deeply rooted in human history across diverse cultures and eras. Herbal face serums are cosmetic products that are formulated for nourishing and treating the skin as they are concentrated skin care treatments with potent plant-based active ingredients rich in antioxidants, vitamins, flavonoids, etc., that penetrate the deeper layer of the skin compared to lotions or creams. It addresses specific skin concerns like fine lines, wrinkles, loss of firmness, dullness and pigmentation thus, boosting the overall confidence of the individual. Studies have shown that oxidative processes may generate unstable free radicals, which in turn initiate harmful chain reactions capable of causing damage to skin cells. Hence, antioxidants are used to counter this. They are responsible

for breaking the chain of radical scavengers and also inhibits the oxidation reaction that can cause damage to the skin. Therefore, natural sources of antioxidants are given greater consideration. Phenolic compounds and flavonoids in the plant materials have been reported as scavengers of free radicals.<sup>[1]</sup> *Bunchosia armeniaca*, commonly known as peanut butter fruit or peanut butter tree is named for its sweet, butter-like texture and flavour of its ripe fruit, is a species in the family Malpighiaceae and is native to northwestern south America. <sup>[2]</sup> It possess antioxidant activity as well as antimicrobial and anti-inflammatory activity. <sup>[3]</sup> *Clitoria ternatea* (Butterfly pea) and *Psidium guajava* (Guava) was used in combination with it for their strong anti-inflammatory and antimicrobial activity respectively which further enhanced the serum. Hence, the purpose of this study is to create an herbal antioxidant face serum using the methanolic fruit extract of *Bunchosia armeniaca* by selecting an optimized batch

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from among the developed formulations by optimization using Box-Behnken design (DoE).

## II. MATERIALS AND METHODS

This *in vitro* study was conducted in practical laboratories of Nazareth College of Pharmacy, Othara, Thiruvalla during the time period from September 2025 to January 2026. The fruits of *Bunchosia armeniaca* was obtained from Eden Garden, Kottayam.

### 2.1 Drug profile of Peanut butter fruit



Figure 1. *Bunchosia armeniaca*

### 2.2 List of ingredients

List of ingredients required for serum: Guava leaves extract, Butterfly pea flower extract, Aloe Vera gel, Sodium Alginate, Sodium Benzoate, Glycerine, Tween 20, Triethanol amine, Sesame oil, Lavender oil, and Distilled water. These chemicals and ingredients were obtained from the practical laboratories of Nazareth College of Pharmacy, Othara, Thiruvalla, 689546.

### 2.3 Method of extraction

The extraction of *Bunchosia armeniaca* fruits was done by using maceration method. To expand the surface area and make it easier to extract the active ingredients, *Bunchosia armeniaca* fruits were first washed, dried, and crushed. Methanol was used as the solvent. In a clean iodine flask,

In traditional medicine, this plant has been used for endocrine, infectious, inflammatory, nutritional and metabolic disorder treatments and some kind of cancer treatment. [4] Active ingredient: Peanut butter Fruit extract. Biological Name: *Bunchosia armeniaca*. Classification: Kingdom- Plantae, Division- Tracheophyta (Vascular plants/Angiosperms), Class- Magnoliopsida (Dicotyledons), Order- Malpighiales, Family- Malpighiaceae (Nance family), Genus- *Bunchosia* and Species- *Bunchosia armeniaca*.

the crushed fruits of *Bunchosia armeniaca* and methanol was added at a ratio of 1:10 (1-part fruits to 10 parts methanol). To ensure that the fruits are evenly distributed throughout the methanol, the mixture is swirled. 48 hours are given for the mixture to stand at room temperature. To stop the active ingredients in the extract from degrading, it was kept in a sterile, airtight container out of the way of sunlight.

### 2.4 Formulation of serum base

Procedure: Sodium alginate was immersed in distilled water over night to form a gel. Triethanolamine was added at the last for desired pH and consistency after adding remaining ingredients one by one in the table given below.

Table – 1 Formulation of serum base (30 ml)

| Ingredient                 | Formulation    |                |                |                |                |                |                |                |                |                 |                 |                 |                 |                 |                 |                 |                 |
|----------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                            | F <sub>1</sub> | F <sub>2</sub> | F <sub>3</sub> | F <sub>4</sub> | F <sub>5</sub> | F <sub>6</sub> | F <sub>7</sub> | F <sub>8</sub> | F <sub>9</sub> | F <sub>10</sub> | F <sub>11</sub> | F <sub>12</sub> | F <sub>13</sub> | F <sub>14</sub> | F <sub>15</sub> | F <sub>16</sub> | F <sub>17</sub> |
| <i>Bunchosia armeniaca</i> | 5.0 ml         | 5.0 ml         | 5.0 ml         | 5.0 ml         | 5.0 ml         | 5.0 ml         | 5.0 ml         | 5.0 ml         | 5.0 ml         | 5.0 ml          | 5.0 ml          | 5.0 ml          | 5.0 ml          | 5.0 ml          | 5.0 ml          | 5.0 ml          | 5.0 ml          |
| <i>Clitoria ternatea</i>   | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml          | 3.5 ml          | 3.5 ml          | 3.5 ml          | 3.5 ml          | 3.5 ml          | 3.5 ml          | 3.5 ml          |
| <i>Psidium guajava</i>     | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml         | 3.5 ml          | 3.5 ml          | 3.5 ml          | 3.5 ml          | 3.5 ml          | 3.5 ml          | 3.5 ml          | 3.5 ml          |
| Aloe vera gel              | 2.0 ml         | 2.0 ml         | 2.0 ml         | 2.0 ml         | 2.0 ml         | 2.0 ml         | 2.0 ml         | 2.0 ml         | 2.0 ml         | 2.0 ml          | 2.0 ml          | 2.0 ml          | 2.0 ml          | 2.0 ml          | 2.0 ml          | 2.0 ml          | 2.0 ml          |
| Sodium benzoate            | 0.4 g          | 0.4 g          | 0.4 g          | 0.4 g          | 0.4 g          | 0.5 g          | 0.5 g          | 0.5 g          | 0.6 g          | 0.6 g           | 0.6 g           | 0.6 g           | 0.6 g           | 0.6 g           | 0.6 g           | 0.6 g           | 0.6 g           |
| Sodium alginate            | 0.3 g          | 0.5 g          | 0.1 g          | 0.1 g          | 0.3 g          | 0.3 g          | 0.5 g          | 0.1 g          | 0.3 g          | 0.3 g           | 0.3 g           | 0.5 g           | 0.5 g           | 0.3 g           | 0.1 g           | 0.5 g           | 0.3 g           |
| Tween 20                   | 1.0 ml         | 1.0 ml         | 1.0 ml         | 1.0 ml         | 1.1 ml         | 1.1 ml         | 1.1 ml         | 1.2 ml         | 1.2 ml         | 1.2 ml          | 1.2 ml          | 1.3 ml          | 1.3 ml          | 1.3 ml          | 1.3 ml          | 0.3 ml          | 0.3 ml          |
| Triethanol amine           | 0.1 ml         | 0.1 ml         | 0.1 ml         | 0.1 ml         | 0.1 ml         | 0.1 ml         | 0.1 ml         | 0.1 ml         | 0.1 ml         | 1.0 ml          | 0.1 ml          | 0.1 ml          | 0.1 ml          | 0.1 ml          | 0.1 ml          | 0.3 ml          | 0.3 ml          |
| Glycerine                  | 2.0 ml         | 2.0 ml         | 2.0 ml         | 2.5 ml         | 3.0 ml         | 3.0 ml         | 3.0 ml         | 3.0 ml         | 3.0 ml         | 3.0 ml          | 3.0 ml          | 3.0 ml          | 3.5 ml          | 3.5 ml          | 3.5 ml          | 0.3 ml          | 0.3 ml          |

|                        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
|------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| <b>Lavender oil</b>    | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml | 0.1 ml |
| <b>Sesame oil</b>      | 4.5 ml | 4.5 ml | 4.5 ml | 4.0 ml | 3.5 ml | 3.5 ml | 3.5 ml | 3.5 ml | 3.5 ml | 3.5 ml | 3.5 ml | 3.5 ml | 3.5 ml | 3.0 ml | 3.0 ml | 3.0 ml | 0.3 ml |
| <b>Distilled water</b> | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   | q.s.   |

### 2.5 Evaluation of the extract

Phytochemical screening of *Bunchosia armeniaca* was done in the practical laboratory of Nazareth College of Pharmacy, Othra to determine the presence of phytochemical constituents present in it.

Test for Alkaloids (Dragendorff's test): To 3 ml of filtrate, add few drops of Dragendorff's reagent. A reddish-brown precipitate was observed. As a result, it suggested the presence of alkaloids. Test for Flavonoids (Shinoda test): To 2 ml of the filtrate, add magnesium turnings followed by conc. HCL. Pinkish red colour was produced. As a result, it suggested the presence of flavonoids. Test for Phenols (Ferric chloride test): To 2 ml of the filtrate, add 2 ml of ferric chloride reagent. A bluish black colour was observed. As a result, it suggested the presence of phenols. Test for Tannins (Lead acetate test): To the extract, add few ml of lead acetate reagent. A white precipitate was formed. As a result, it suggested the presence of tannins. Test for Carbohydrates (Molisch test): To the extract, add Molisch reagent and add few drops of conc. sulphuric acid along the sides of the test tube. A violet ring was formed at the junction of the two liquids. As a result, it suggested the presence of carbohydrates.

### 2.6 Evaluation of the face serum

In vitro study; Organoleptic evaluation: The face serum was visually evaluated for colour, odour, state and texture. pH determination: The pH of the sample was evaluated using a digital pH meter. The instrument was first calibrated using neutral buffer solution (pH 7.01) and acid buffer solution (pH 4.01). Then the electrodes were washed with distilled water and dried with a tissue.<sup>[5]</sup> Viscosity determination: The apparatus used was Brookfield viscometer. At 60 rpm, spindle no. 27 was used and each reading of the sample were taken after equilibrium was attained. The viscosity was measured in cps. Spreadability test: It is expressed in terms of time in seconds. A drop of sample was placed between two glass slides of standard dimensions under a certain load i.e., a weight was placed on the upper slide. This presses the sample uniformly to form a thin layer. The length of this layer was measured. The weight was then removed and the excess sample adhering to the slides was scraped off. The time taken by the upper slide to slip off was noted.<sup>[6]</sup>

$$\text{Spreadability} = \frac{m \times l}{t} \quad (1)$$

Where, m – standard weight (50g), l – length, and t – time (seconds). Stability Studies: This was carried out based on ICH- recommended conditions. In a stability chamber, sufficient quantity of the sample was stored in a well closed-glass vial and subjected to short-term stability studies for a period of one month maintaining a

temperature of  $40 \pm 2^\circ\text{C}$  and relative humidity of  $75 \pm 5\%$  RH. Homogeneity: It was evaluated by placing two drops of sample on a glass slide. Another glass slide was placed over it. The homogenous arrangement was observed. Phase separation: At room temperature, the sample was kept in a closed container away from sunlight. After 24 hours, it was observed for any phase separation. Ash value: The sample was taken in a silica crucible and weighed. It was then evaporated to dryness and incinerated gradually at  $450-600^\circ\text{C}$  until ash is obtained. After it was cooled in the desiccator, its weight was taken. The ash value was calculated with respect to the initial weight of the sample. The residue left represents the total inorganic content present in the sample.

$$\text{Ash content (\%)} = \left( \frac{\text{Weight of Ash}}{\text{Initial weight of sample}} \right) \times 100 \quad (2)$$

Redispersion test: It is performed by transferring the sample into a clean and dry centrifuge tube. At a specified speed and time, the sample was centrifuged to induce sedimentation. Afterwards, the supernatant was carefully removed. The sediment is redispersed by gentle shaking or inversion for a fixed period. The sample is then visually examined for uniformity, absence of aggregates and ease of redispersion. Anti-microbial activity: Microbial analysis is usually carried out to ensure the safety of the formulation for customers to use and confirm hygienic and high-quality handling. The entire surface of the agar plate was inoculated by spreading a volume of the microbial inoculum (*Staphylococcus aureus*). A hole of diameter, 6-8 mm is punched aseptically with a sterile cork tip and a small volume of serum is introduced into the well. Then the agar plates were incubated at  $37^\circ\text{C}$  for 12 hours. *In vitro* Antioxidant activity (by DPPH assay): In this method, the stable free radical DPPH reacts with the antioxidant compound. After the addition of an antioxidant, the decrease in the absorption of the DPPH solution was measured at 520 nm. The reduction in colour intensity was also measured. Plates were removed from the incubation time and checked for microbial growth by comparing them to the control. Principle: The free radical scavenging capacity of the extracts were evaluated using the DPPH radical scavenging assay. This assay is based on the ability of plant extracts to donate hydrogen atoms, which is determined by measuring the reduction in colour intensity of a methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). In the presence of antioxidants, the initially violet or purple DPPH solution is reduced to yellow, indicating radical scavenging activity. Procedure: A DPPH solution of  $6 \times 10^{-5}$  M was prepared in methanol by dissolving 7.89 mg of DPPH in 100 ml of solvent. For each test sample, 500  $\mu\text{L}$  of the sample solution was transferred into Eppendorf tubes, and each concentration

was tested in triplicate. Subsequently, 500  $\mu$ L of the DPPH solution was added to each tube and mixed thoroughly. The reaction mixture was incubated at room temperature for half an hour in the dark. Absorbance was then measured at 520 nm using a UV-visible

$$\text{Percentage of Inhibition (\%)} = \frac{(\text{absorbance of DPPH blank} - \text{True absorbance})}{\text{Absorbance of DPPH blank}} \times 100 \quad (3)$$

**In vitro** Anti-inflammatory activity: It was evaluated using egg albumin denaturation method. Principle: This method evaluates whether a substance can prevent or reduce protein denaturation. Egg albumin (egg white) is used as a model protein, which denatures when exposed to heat, pH changes, or chemical agents. Protein denaturation involves structural changes that lead to loss of biological function and is associated with inflammation and tissue damage. Compounds with anti-inflammatory properties can stabilize protein structure and inhibit denaturation. In this assay, different concentrations of a test compound are mixed with egg albumin under controlled conditions, and denaturation is induced. The degree of denaturation is measured by absorbance, and the percentage inhibition is calculated. Substances that significantly reduce egg

$$\text{Percentage of Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100 \quad (4)$$

spectrophotometer. Ascorbic acid was used as the positive control, while distilled water served as the negative control. The percentage inhibition of DPPH radicals relative to the standard was calculated using the formula below, and the  $IC_{50}$  values were subsequently determined.

albumin denaturation are considered to have potential anti-inflammatory activity. Procedure: A reaction mixture was prepared by combining 0.5 ml of HAFS with 0.45 ml of a 5% bovine egg albumin solution. The initial pH of the mixture was adjusted to 6.3 using 0.1 N HCL. The mixture was then incubated at 37°C for 20 minutes, followed by heating at 57°C for 30 minutes to induce protein denaturation. After cooling, the samples were transferred into 96 well plates, and the absorbance was measured at 660 nm using a microplate reader. Diclofenac sodium (1000  $\mu$ g/ml) was used as the standard, while water (0.05 ml) served as the control. The percentage inhibition of egg albumin denaturation was calculated using the equation given below.

## 2.7 Experimental Design

Optimization is the process of fine-tuning product variables to achieve the most desirable outcome. It helps us to achieve the best balance of safety, performance and enjoyable texture of the cosmetic product. This makes it satisfying for the customer to use.

Box-Behnken Design (BBD) is one of the commonly used DoE-based Response Surface Methodology (RSM) used for optimization of formulations and processes in pharmaceutical technology. It evaluates the relationship between independent variables (factors) and dependent responses using a quadratic model, while requiring fewer experimental runs than full factorial designs.

## BOX-BEHNKEN DESIGN

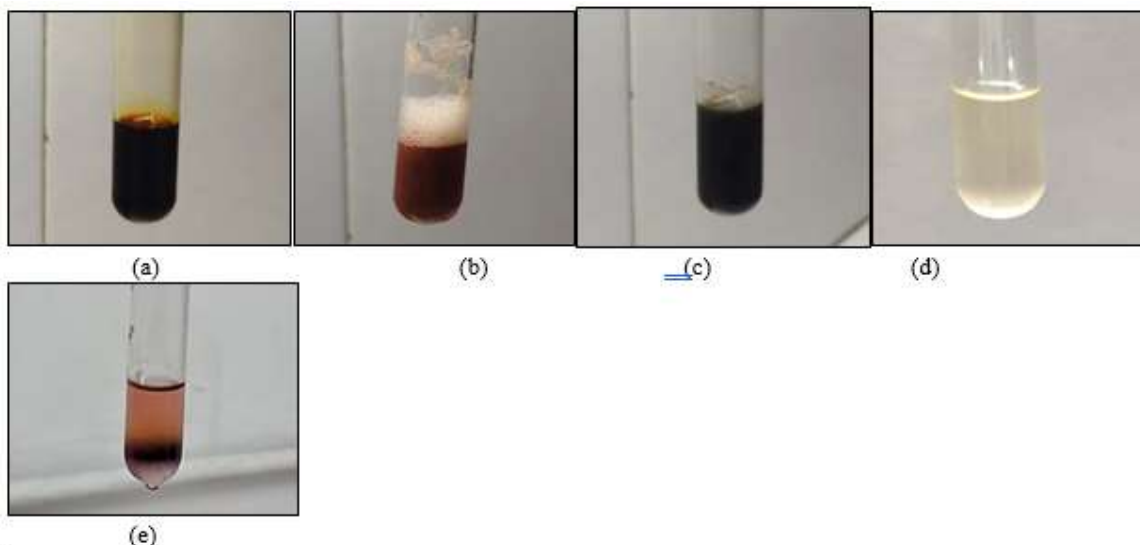
Table – 2 Optimization of Herbal Antioxidant Face Serum

| Std | Run | Factor1<br>A: rpm (m/s) | Factor 2<br>B: conc. of thickening<br>agent (%w/w) | Factor 3<br>C: pH | Response 1<br>Spreadability<br>(g.cm/s) | Response 2<br>Viscosity (Pa. S) |
|-----|-----|-------------------------|----------------------------------------------------|-------------------|-----------------------------------------|---------------------------------|
| 5   | 1   | 700                     | 0.3                                                | 4.7               | 31.6                                    | 1085                            |
| 12  | 2   | 750                     | 0.5                                                | 5.2               | 32.5                                    | 1020                            |
| 2   | 3   | 800                     | 0.1                                                | 5.4               | 36.8                                    | 960                             |
| 1   | 4   | 700                     | 0.1                                                | 5.3               | 33.85                                   | 970                             |
| 15  | 5   | 750                     | 0.3                                                | 4.9               | 34.38                                   | 1020                            |
| 13  | 6   | 750                     | 0.3                                                | 5.5               | 34                                      | 1024                            |
| 4   | 7   | 800                     | 0.5                                                | 5.24              | 34.9                                    | 1010                            |
| 9   | 8   | 750                     | 0.1                                                | 5.22              | 35                                      | 950                             |
| 7   | 9   | 700                     | 0.3                                                | 4.57              | 34.2                                    | 1100                            |
| 14  | 10  | 750                     | 0.3                                                | 5.5               | 34.6                                    | 1090                            |
| 6   | 11  | 800                     | 0.3                                                | 5.58              | 34.38                                   | 1080                            |
| 3   | 12  | 700                     | 0.5                                                | 4.8               | 33.85                                   | 1030                            |
| 10  | 13  | 750                     | 0.5                                                | 4.5               | 33.28                                   | 1025                            |
| 8   | 14  | 800                     | 0.3                                                | 5.5               | 34.5                                    | 1020                            |
| 11  | 15  | 750                     | 0.1                                                | 5.3               | 31.5                                    | 955                             |
| 15  | 16  | 700                     | 0.5                                                | 5.0               | 31.9                                    | 1030                            |
| 14  | 17  | 800                     | 0.3                                                | 5.38              | 34.2                                    | 1025                            |

### III. RESULTS AND DISCUSSIONS

#### 3.1 Phytochemical screening

Preliminary phytochemical screening for *Bunchosia armeniaca* was performed and the results are as follows.



**Figure 2.** (a) Dragendorff's test (b) Shinoda test (c) Ferric chloride test (d) Lead acetate test (e) Molisch test

#### 3.2 Experimental Design

##### ANOVA FOR QUADRATIC MODEL – SPREADABILITY

##### Response 1: Spreadability

**Table – 3** Anova for Spreadability

| Source                        | Sum of Squares | df | Mean Square | F-value | P-value |                 |
|-------------------------------|----------------|----|-------------|---------|---------|-----------------|
| <b>Model</b>                  | 16.05          | 9  | 1.78        | 5.96    | 0.0318  | Significant     |
| A – rpm                       | 0.1229         | 1  | 0.0776      | 0.4107  | 0.5498  |                 |
| B – conc. of thickening agent | 0.076          | 1  | 0.0776      | 0.2596  | 0.6321  |                 |
| C – pH                        | 3.97           | 1  | 3.97        | 13.27   | 0.0149  |                 |
| AB                            | 0.1440         | 1  | 0.1440      | 0.4815  | 0.5186  |                 |
| AC                            | 0.3426         | 1  | 0.3426      | 1.15    | 0.3334  |                 |
| BC                            | 2.48           | 1  | 2.48        | 8.31    | 0.0345  |                 |
| A <sup>2</sup>                | 0.0016         | 1  | 0.0016      | 0.0054  | 0.9444  |                 |
| B <sup>2</sup>                | 2.43           | 1  | 2.43        | 8.14    | 0.0357  |                 |
| C <sup>2</sup>                | 6.72           | 1  | 6.72        | 22.48   | 0.0051  |                 |
| <b>Residual</b>               | 1.50           | 5  | 0.2991      |         |         |                 |
| Lack of Fit                   | 1.32           | 4  | 0.3289      | 1.83    | 0.4995  | Not significant |
| Pure error                    | 0.1800         | 1  | 0.1800      |         |         |                 |
| <b>Cor Total</b>              | 17.54          | 14 |             |         |         |                 |

**Table – 4** Lack of Fit of Spreadability

| Source           | Sum of Squares | df       | Mean Square   | F-value     | p-value       |                  |
|------------------|----------------|----------|---------------|-------------|---------------|------------------|
| Linear           | 16.77          | 10       | 1.68          | 9.32        | 0.2501        |                  |
| 2FI              | 8.37           | 7        | 1.20          | 6.65        | 0.2904        |                  |
| <b>Quadratic</b> | <b>1.32</b>    | <b>4</b> | <b>0.3289</b> | <b>1.83</b> | <b>0.4995</b> | <b>Suggested</b> |
| Cubic            | 0.0000         | 0        |               |             |               | <b>Aliased</b>   |
| Pure Error       | 0.1800         | 1        | 0.1800        |             |               |                  |

**Table – 5** Fit Summary of Spreadability

| Source | Sequential p-value | Lack of fit p-value | Adjusted R <sup>2</sup> | Predicted R <sup>2</sup> |  |
|--------|--------------------|---------------------|-------------------------|--------------------------|--|
| Linear | 0.9408             | 0.2501              | -0.2295                 | -1.1292                  |  |
| 2FI    | 0.1230             | 0.2904              | 0.1469                  | -0.9422                  |  |

|                  |               |               |               |                |                  |
|------------------|---------------|---------------|---------------|----------------|------------------|
| <b>Quadratic</b> | <b>0.0244</b> | <b>0.4995</b> | <b>0.7613</b> | <b>-0.0925</b> | <b>Suggested</b> |
| Cubic            | 0.4995        |               | 0.8564        |                | <b>Aliased</b>   |

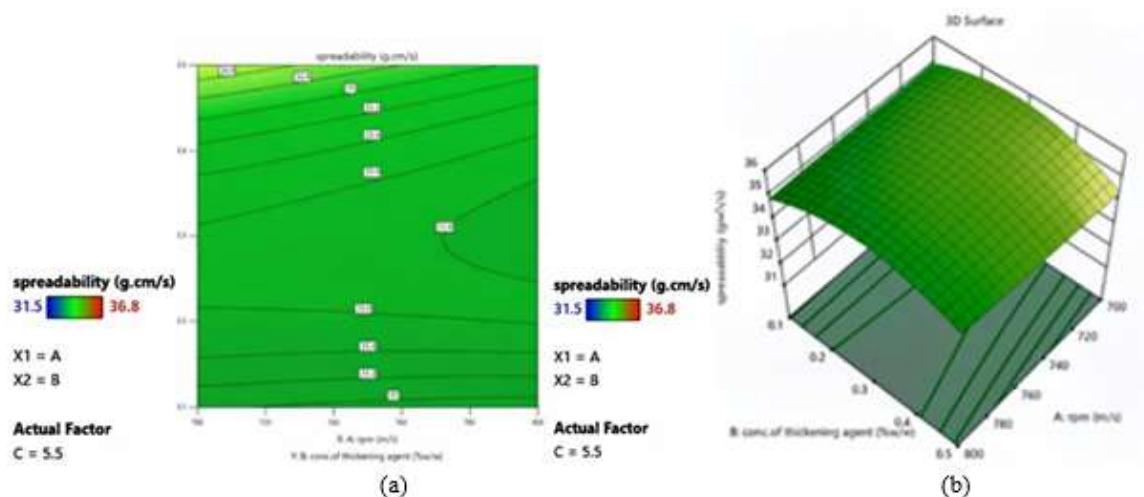


Figure 3. (a) Contour Plot of Spreadability (b) 3D Surface Plot of Spreadability

**Response 2: Viscosity****Table – 6 Anova for Viscosity**

| Source                        | Sum of squares | df | Mean Square | F-value | P-value |                 |
|-------------------------------|----------------|----|-------------|---------|---------|-----------------|
| <b>Model</b>                  | 46310.36       | 9  | 5145.60     | 112.56  | <0.0001 | significant     |
| A – rpm                       | 198.63         | 1  | 198.63      | 4.34    | 0.0915  |                 |
| B – conc. of thickening agent | 7735.87        | 1  | 7735.87     | 169.22  | <0.0001 |                 |
| C – pH                        | 140.95         | 1  | 140.95      | 3.08    | 0.1395  |                 |
| AB                            | 7.64           | 1  | 7.64        | 0.1671  | 0.6996  |                 |
| AC                            | 9.39           | 1  | 9.39        | 0.2054  | 0.6694  |                 |
| BC                            | 22.64          | 1  | 22.64       | 0.4954  | 0.5130  |                 |
| A <sup>2</sup>                | 43.13          | 1  | 43.13       | 0.9435  | 0.3760  |                 |
| B <sup>2</sup>                | 30800.94       | 1  | 30800.94    | 673.76  | <0.0001 |                 |
| C <sup>2</sup>                | 10.41          | 1  | 10.41       | 0.2276  | 0.6534  |                 |
| <b>Residual</b>               | 228.57         | 5  | 45.71       |         |         |                 |
| Lack of Fit                   | 226.57         | 4  | 56.64       | 28.32   | 0.1399  | not significant |
| Pure Error                    | 2.00           | 1  | 2.00        |         |         |                 |
| <b>Cor Total</b>              | 46538.93       | 14 |             |         |         |                 |

**Table – 7 Lack of Fit of Viscosity**

| Source           | Sum of Squares | df       | Mean Square  | F-value      | p-value       |                  |
|------------------|----------------|----------|--------------|--------------|---------------|------------------|
| Linear           | 38127.96       | 10       | 3812.80      | 1906.40      | 0.0178        |                  |
| 2FI              | 36978.61       | 7        | 5282.66      | 2641.33      | 0.0150        |                  |
| <b>Quadratic</b> | <b>226.57</b>  | <b>4</b> | <b>56.64</b> | <b>28.32</b> | <b>0.1399</b> | <b>Suggested</b> |
| Cubic            | 0.0000         | 0        |              |              |               | <b>Aliased</b>   |
| Pure Error       | 2.00           | 1        | 2.00         |              |               |                  |

**Table – 8 Fit Summary of Viscosity**

| Source           | Sequential p-value | Lack of Fit p-value | Adjusted R <sup>2</sup> | Predicted R <sup>2</sup> |                  |
|------------------|--------------------|---------------------|-------------------------|--------------------------|------------------|
| Linear           | 0.5151             | 0.0178              | -0.0428                 | -0.6099                  |                  |
| 2FI              | 0.9675             | 0.0150              | -0.3906                 | -3.1058                  |                  |
| <b>Quadratic</b> | <b>&lt;0.0001</b>  | <b>0.1399</b>       | <b>0.9862</b>           | <b>0.9086</b>            | <b>Suggested</b> |
| Cubic            | 0.1399             |                     | 0.9994                  |                          | <b>Aliased</b>   |

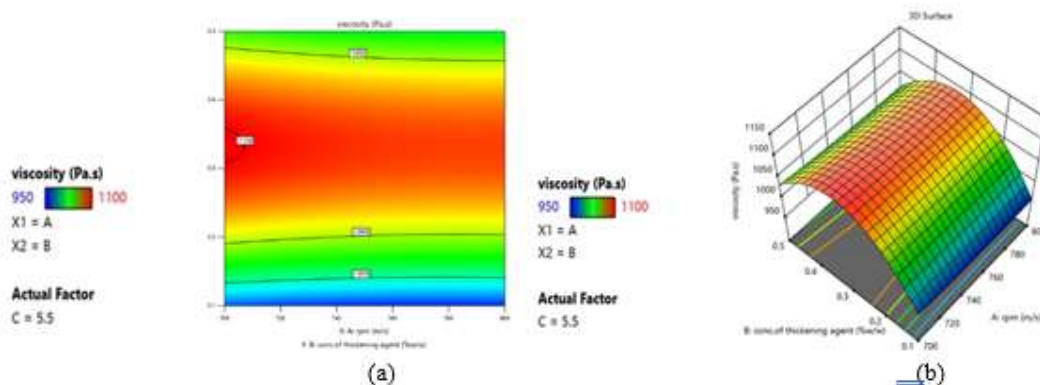


Figure 4. (a) Contour Plot of Viscosity (b) 3D Surface Plot of Viscosity

### 3.3 Evaluation of the face serum

Table – 9 Organoleptic evaluation

| Optimized formulation | Physical parameters |             |                |        |
|-----------------------|---------------------|-------------|----------------|--------|
|                       | Texture             | Colour      | Odour          | State  |
| F <sub>8</sub>        | Smooth              | Coral green | Characteristic | Liquid |



Figure 5. (a) Organoleptic evaluation (b) Formulation of the face serum

Table – 10 Data of the Evaluation parameters

| Parameters        | Optimized formulation – F <sub>8</sub> | Mean±Standard Deviation |
|-------------------|----------------------------------------|-------------------------|
| pH                | Good                                   | 5.22±0.02               |
| Viscosity         | Good                                   | 950±0.2 (cPs)           |
| Spreadability     | Good                                   | 35±0.025166 (g.cm/sec)  |
|                   |                                        | <b>Conclusion</b>       |
| Homogeneity       | Good                                   | Good                    |
| Ash value         | Low ash value                          | Good                    |
| Redispersion test | Redispersed easily                     | Good                    |
| Phase separation  | No change                              | Good                    |
| Stability studies | Slight changes                         | Good                    |

### 3.4 Antioxidant activity – in vitro (using DPPH assay)

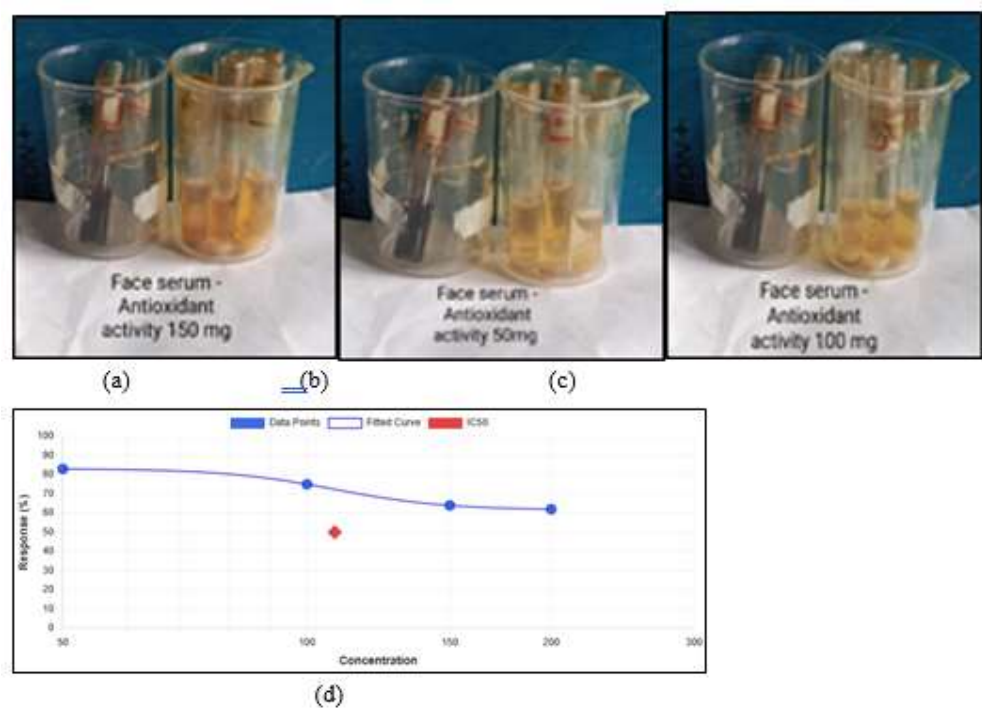
Herbal antioxidant face serum extract has shown *in vitro* antioxidant activity by DPPH assay in the current investigation.

At 50 mg/ml of the herbal face serum, the inhibition percentage is 83% and the IC<sub>50</sub> value was found to be

108.20 mg/ml compared to the standard ascorbic acid IC<sub>50</sub> value of 33.7334 mg/ml. As a result, the antioxidant activity of the herbal face serum is good compared to vitamin C (standard ascorbic acid). All experiments were done in triplicates.

**Table – 11** Herbal Face Serum (HFS) antioxidant IC<sub>50</sub> value (mg/ml) compared to standard vit. C IC<sub>50</sub> value (mg/ml)

| SL.NO                            | Concentration (mg) | Average | IC <sub>50</sub> value |
|----------------------------------|--------------------|---------|------------------------|
| 1                                | 50                 | 83 %    | 108.20 mg/ml           |
| 2                                | 100                | 75 %    |                        |
| 3                                | 150                | 64 %    |                        |
| Standard Ascorbic acid vitamin C |                    |         |                        |
| 1                                | 50 mg              | 91%     | 33.7334 mg/ml          |
| 2                                | 100 mg             | 86%     |                        |
| 3                                | 150 mg             | 86%     |                        |
| 4                                | 200 mg             | 92%     |                        |
| 5                                | 250 mg             | 84%     |                        |



**Figure 6.** (a)Antioxidant activity – 50 mg (b)Antioxidant activity – 100 mg (c)Antioxidant activity – 150 mg  
(d)Graphical representation depicting DPPH radical scavenging assay of sample

**3.5 Anti-microbial activity (Agar well diffusion method)**

**Table – 12** Anti-microbial activity

| SL.NO. | ZONE OF INHIBITION OF STANDARD TETRACYCLINE (mm) | OPTIMIZED FORMULATION | ZONE OF INHIBITION (mm) |
|--------|--------------------------------------------------|-----------------------|-------------------------|
| 1      | 16.0                                             | F <sub>8</sub>        | 20.0                    |



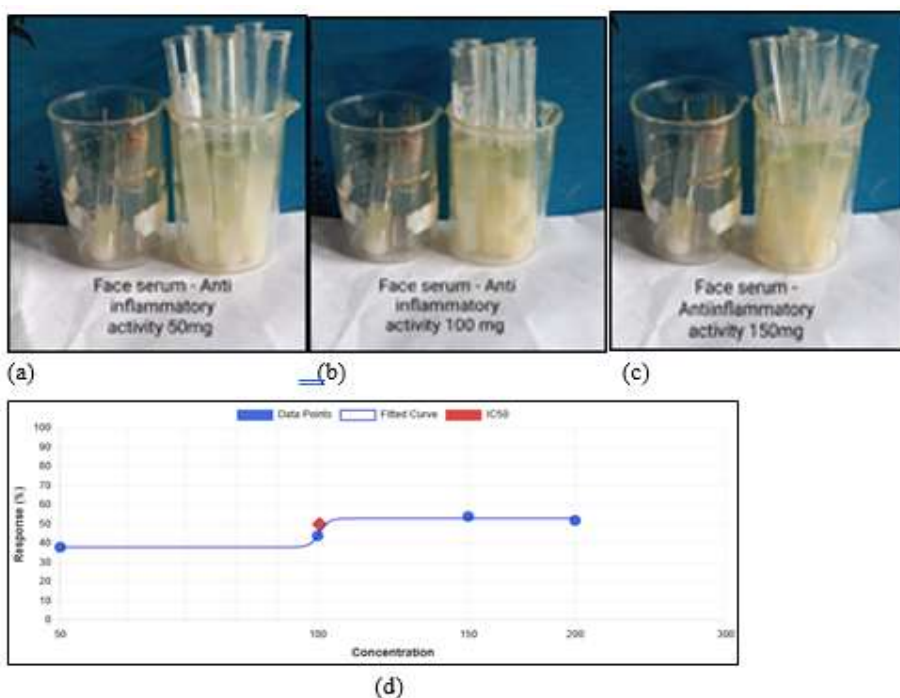
**Figure 7.** Anti-microbial activity  
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The anti-microbial activity of the optimized formulation, F<sub>8</sub> was evaluated using tetracycline as the control against *Staphylococcus aureus*. The formulation exhibited a zone of 20 mm, indicating its anti-microbial activity against the bacterium.

### 3.6 Anti-inflammatory activity – *in vitro* (egg albumin denaturation method)

**Table – 13** Herbal Face Serum (HFS) *in vitro* anti-inflammatory activity

| SL.NO                      | Concentration (mg) | Average | IC <sub>50</sub> value |
|----------------------------|--------------------|---------|------------------------|
| 1                          | 50                 | 38%     | 100.51<br>mg/ml        |
| 2                          | 100                | 44%     |                        |
| 3                          | 150                | 54%     |                        |
| Standard Diclofenac sodium |                    |         |                        |
| S.NO                       | Concentration (mg) | Average | IC <sub>50</sub> value |
| 1                          | 50 mg              | 61%     | 121.29<br>mg/ml        |
| 2                          | 100 mg             | 75%     |                        |
| 3                          | 150 mg             | 85%     |                        |



**Figure 8.** (a)Anti-inflammatory activity – 50 mg (b)Anti-inflammatory – 100 mg (c)Anti-inflammatory – 150 mg (d)Graphical representation depicting anti-inflammatory activity of the sample

At 50 mg/ml, the herbal face serum has an inhibition percentage of 38% and the IC<sub>50</sub> value was found to be 100.51 mg/ml. This was compared with the IC<sub>50</sub> value of the standard diclofenac sodium, 121.29 mg/ml. Herbal face serum exhibits greater anti-inflammatory efficacy compared with the standard diclofenac sodium.

#### IV. CONCLUSION

Herbal antioxidant face serum containing *Bunchosia armeniaca* in combination with *Clitoria ternatea* and *Psidium guajava* was successfully formulated. The study has shown that the face serum has maximum antioxidant activity which can treat skin problems like aging,

wrinkles, hyper-pigmentation, etc., along with anti-microbial and anti-inflammatory activity. A total of seventeen runs were generated using DoE software. From the generated data, the optimized formulation was found to be F<sub>8</sub>. Natural ingredients were used to prepare the formulations. Hence, they are considered safe for regular use which is an advantage over synthetic face serums in the market. In conclusion, the findings highlight the herbal antioxidant face serum's efficacy for skin care applications and indicate substantial application for potential for improvement and expansion.

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