IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
May 2024 Vol.:30, Issue:5

Formulation and Evaluation of a Polyherbal Anti-Aging Face Serum

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ISSN 2349-7203
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Submitted: 27 April 2024
Accepted: 02 May 2024
Published: 30 May 2024

Keywords: Anti-aging, Herbal face serum, Liquorice, Manjistha, Carrot seed oil, Almond oil, Aloe Vera

ABSTRACT

As the demand for natural and effective anti-aging skincare products continues to rise. This study focuses on the development and evaluation of an anti-aging herbal face serum utilizing a synergistic blend of botanical ingredients known for their rejuvenating and skin-nourishing properties. The formulated serum incorporates powder extract of liquorice (*Glycyrrhiza glabra*) and manjistha (*Rubia cordifolia*), along with essential oils of carrot seed (*Daucus carota*) and almond (*Prunus dulcis*), enriched with the relaxing and hydrating activity of aloe vera (*Aloe barbadensis*), rose water, and glycerin. The serum was prepared using standard laboratory procedures, ensuring proper emulsification and stability. The evaluation of the formulated serum included various physicochemical tests to assess its appearance, pH, and stability over time. Preliminary results indicate that the formulated anti-aging herbal face serum exhibits desirable physicochemical properties, including a smooth texture, suitable viscosity, and stable emulsion. Overall, this study presents a promising approach to harnessing the beneficial activity of botanical extracts and oils in the development of effective anti-aging skincare formulations. Additional investigation is acceptable to explore the clinical efficiency and safety of the formulated serum in human trials, paving the way for its potential incorporation into skincare regimens aimed at promoting youthful and radiant skin.

INTRODUCTION

Herbal extracts, as the name suggests, are derived from herbs with historical references found in the Bible, Vedas, and Unani scriptures. The term "herbal cosmetics" refers to products formulated using various allowable cosmetic ingredients to outline the pedestal in which one or more herbal ingredients are worn to afford defined cosmetic benefits[1]. The remark cosmetic was resultant from the Greek word "kosmeticos" meaning having the supremacy, arrangement, and expertise in embellishment. The beginning of cosmetics has been allied to hunting, fighting, belief, and superstition and was later on associated with medicine[2].

There is a growing interest in using herbal remedies to supplement allopathic treatments, which are the only treatments used in traditional medicine. Understanding how outside substances impact our bodies requires an understanding of human skin, especially in the fields of dermatology, toxicology, pharmacology, and cosmetology. Since prehistoric times, humans have been drawn to beauty and wellness, and they still look for ways to seem young and well. The Food Drug & Cosmetic Act defines cosmetics as anything applied to the human body that have the purpose of cleansing, beautifying, enhancing attractiveness, or altering looks. Cosmetology is the learning of cosmetics and their uses as well as the art of improving nails, hair, and skin through aesthetic procedures. Strong active ingredients are infused extremely into the skin by a superior face serum, which doesn't need harsh chemicals to show effects right away. A significant portion of cosmetic goods sold globally are skin-lightening solutions, which provide the promise of perfect skin free from scars, blemishes, and aging spots[3].

The need to lighten overall skin tone and get rid of localized hyperpigmentation is what drives the market for "skin fairness products." Citizens in Western nations covet to get relieve of or stop the expansion of irregular pigmentation, such as freckles (Lentigo aestiva), liver spots (Lentigo senilis), melisma, age spots, or liver spots (connected to sun damage or aging and every so often emerge as raised spots or Seborrheic keratoses). Lighter skin tones are linked to beauty and nobility in Asia[4]. Consequently, the goal of using skin-lightening products in Asian nations is to lighten and brighten the skin tone. As much as 10% of Melanin, a dark pigment, is produced by skin cells in the epidermis' deepest layer. The real color of the skin is determined by the kind, quantity, and distribution pattern of melanin formed by the melanocyte inside the surrounding keratinocytes. In the occurrence of the

enzyme tyrosinase, a sequence of oxidative events involving the amino acid tyrosine results in the progress of melanin[5].

Face serums come in water-and oil-based formulations and they usually include ten times more biologically active ingredients than standard creams. Because face serum's tiny molecular structure allows for fast penetration into the skin, people may notice noticeable improvements in as little as one month when adding a only some drops of the product to their regular skincare routine[6].Face serums frequently include nutrients and useful active ingredients such ceramides, amino acids, antioxidants, and other necessary substances. Licorice extract (Mulethi), manjistha (*Rhus cordifolius* plant extract), carrot seed oil, flaxseed gel, aloe vera gel, almond oil, glycerin, and salicylic acid as a preservative are among the ingredients frequently used in herbal cosmetics. Skincare products are designed to appeal to different types of skin. Oily and combination skin can benefit from gel and liquid preparations, while normal to dry skin can benefit from serums and lighter lotions[7]. Dry to extremely dry skin can benefit from more emollient lotions and moisturizing creams. Even though different products have different textures, the basic elements needed for healthy skin are always present. Finally, even though our skin, which is the prevalent organ in the body, constantly tries to heal and mend itself, there are times when it has dry patches because of things like pollution, UV rays, and poor makeup removal. Face serums with components that have been connected to better barrier function and a decrease in fine lines, blemishes and wrinkles. Plant-based herbal extracts can be traced back to ancient scriptures such as the Bible, Vedas, and Upanishads. There is a growing interest in using herbal remedies to supplement allopathic treatments, which are the only treatments used in traditional medicine. Understanding how outside substances impact our bodies requires an understanding of human skin, especially in the fields of dermatology, toxicology, pharmacology, and cosmetology. Since prehistoric times, humans have been drawn to beauty and wellness, and they still look for ways to seem young and well[8].

a. Liquorice Root Extract for Skin:

Liquorice roots, specifically the extract from *Glycyrrhiza glabra*, offer numerous profit for the skin due to its antioxidant properties, particularly from compounds like glycyrrhizin, triterpene saponins, and flavonoids. These components contribute to various skin-enhancing effects such as skin whitening, depigmentation, skin lightening, anti-aging properties, anti-

inflammatory effects, moisturizing benefits, acne-fighting capabilities, and photoprotective effects[9].

By incorporating liquorice root extract into your skincare practice have shown benefit from its diverse range of skin-improving properties. It brightens your complexion, encounter signs of aging, relieve inflammation, or protect your skin from UV damage; liquorice root extract can be a valuable addition to your skincare regimen. It provides UVB protection and acts as a moisturizing agent[10].

b. Aloe Vera Gel for Radiation Protection and Skincare

Aloe Vera is used against skin damage caused by exposure to ultraviolet (UV) and gamma radiation. Its protective qualities include:

- Anti-radiation effects: It has demonstrated the capacity to mitigate skin damage resulting from radiation exposure.

- Moisturizing and anti-aging properties: Aloe Vera gel contains muco polysaccharides, which bind moisture into the skin, contributing to its moisturizing abilities. Furthermore, aloe stimulates fibroblasts, promoting the production of collagen and elastin fibers, leading to increased skin elasticity and reduced wrinkling[11].

c. Carrot Seed Oil: A Natural Solution for Youthful Skin and Oil Regulation

- Anti-aging properties: This essential oil is recognized for its ability to reverse and diminish wrinkles, and boost appearance.

- Ideal for oily skin: It helps to control oil discharge, maintaining a balanced and healthy complexion.

- Rich in carotenoids: Carrot seed oil contains potent antioxidants that have demonstrated anti-aging, anti-degenerative, and potential anti-cancer effects.

- Cosmetic rejuvenation: A 2019 study highlighted the rejuvenating properties of oil at a cosmetic level[12].

d. Manjistha: A Potent Herb for Skin Enhancement

Manjistha, also known as Indian Madder, is a herb with a rich history in Ayurveda, the ancient Indian system of medicine. Derived from the dried roots of the *Rubia Cordifolia*

plant, Manjistha has been widely used for its skin-enhancing properties. This potent herb is known to address various skin concerns, including acne, pigmentation, and inflammation[13]. Regular use of it can facilitate fade hyperpigmentation, revealing a more even and glowing complexion[14].

e. Flaxseed or Linseed Gel: A Nutrient-Packed Skincare Treatment

Derivative from the flax plant (*Linum usitatissimum*), belonging to the Linaceae family, flaxseed is renowned for its nutritional benefits and emerging skincare applications. Flaxseed is a rich source of dietary fiber, omega-3 fatty acids (including alpha-linolenic acid), and phytoestrogens called lignans. The fiber in flaxseed primarily resides within the seed coat[15].

Flaxseed gel offers a variety of skincare benefits, including:

- Hydration: Due to its high water content, flaxseed gel can help replenish and lock in moisture, creating a dewy and supple complexion.

- Reduced inflammation: Phytoestrogens present in flaxseed gel possess anti-inflammatory properties, which can aid in calming irritated skin[16].

- Improved skin barrier function: The presence of omega-3 fatty acids in flaxseed gel supports the maintenance of a healthy skin barrier, reducing trans epidermal water loss and enhancing overall skin hydration.

By incorporating flaxseed gel into your skincare habitual, it experiences the numerous benefits this nutrient-packed ingredient has to offer[17].

f. Almond Oil: A Nutty and Versatile Oil for Skincare

It is also known as *Oleum amygdalae*, is a glyceryl oleate with a nutty taste and a slight odor. It is almost insoluble in alcohol but readily soluble in chloroform or ether, making it a popular substitute for olive oil. Almond oil offers numerous benefits for your skin, including:

- Moisturization: Almond oil acts as an emollient, helping to lock in moisture and keep your skin hydrated.

- Anti-inflammatory properties: Almond oil contains anti-inflammatory compounds that can help soothe irritated skin.

- Skin-nourishing vitamins: it is rich in vitamins E and A, which can help improve skin health and reduce the signs of aging[18].

g. Glycerin

Glycerin is a safe and effective substance for use on the face, according to scientific research. It aid as a humectant, allowing the skin to maintain moisture, which can amplify skin hydration, relieve dryness, and refresh the skin's surface[19].

h. Rose Water: A Natural Skin Toner with pH-Balancing Properties

Rose water is a herbal skin toner with pH-balancing properties. Incorporating almond oil, glycerin, and rose water into your skincare everyday can afford numerous benefits, including moisturization, anti-inflammatory properties, skin nourishment, hydration, and pH-balancing effects[20].

MATERIALS AND METHODS

Materials

Plant Materials: The following plant resources were collected from the backyard and authenticated by the botanist. The materials were dried in shade and powdered.

Table 1: List of ingredients used in formulation with their uses

S.no.	Ingredients	Collection	Category
1.	Liquorice	Local Market	Depigmentation& Anti-oxidant
2.	Manjistha	Local Market	Skin-brightening, Anti-bacterial & Anti-oxidant
3.	Carrot Seed Oil	Local Market	Anti-aging
4.	Almond Oil	Local Market	Anti-inflammatory
5.	Flax Seed	Devsthali Vidyapeeth College Of Pharmacy	Hydration & Anti-inflammatory
6.	Rose Water	Devsthali Vidyapeeth College Of Pharmacy	Anti-inflammatory
7.	Aloe Vera	Devsthali Vidyapeeth College Of Pharmacy	Anti-bacterial
8.	Glycerin	Devsthali Vidyapeeth College Of Pharmacy	Hydration

Extraction of active constituents

Method of extraction of licorice

After purchasing dried licorice roots from a nearby market, the entire root was cleaned and allowed to dry in the shade for three to four days. In a grinder, the roots were cut into tiny pieces and position into a fine powder. 500 ml of ethanol and water (1:1) were added to a round-bottom flask after 50 g of powder had been weighed, packed into a muslin cloth bag, and placed within the Soxhlet extractor's body. The Soxhlet extractor, round-bottom flask, and condenser were then installed on the apparatus with the use of clamps and a stand. For a constant water flow, the condenser was fitted with a rubber tube that was connected to the tap water. The isomantle was used to heat the solvent, causing it to start evaporating and flow through the device and into the condenser. The cycle restarted when the solvent level reached the siphon and emptied back into the flask. The procedure was designed to run for six hours in total. Ultimately, the extract was filled the flask with a circular bottom. After the procedure was complete, a little yield of extracted plant material (about 2-3 ml) was left in the glass bottom flask after the solvent was evaporated using a rotary evaporator at 40°C. The extract was stored in a porcelain bowl until all of the ethanol had evaporated[21].

Method of extraction of Aloe Vera gel

It is collected through mechanical homogenization at 1,200 RPM. After homogenization, the gel is filtered using a vacuum filtration system to obtain clear and transparent gel. Incorporating gel into your skincare schedule can offer multiple benefits, including enhanced moisture retention, improved skin elasticity, and potential protection against radiation damage[22].

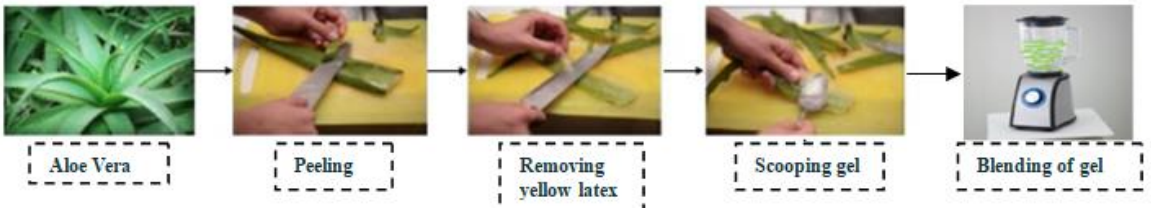

Figure 1: Method of extraction of Aloe Vera gel

Carrot Seeds Oil Extraction Process

Essential Oil Recovery Method:

Dried carrot seeds were grounded into small pieces and subjected to hydro distillation by means of Clevenger-type equipment for 4 hours. The obtained oils were dried in excess of anhydrous sodium sulfate, resulting in an essential oil yield of approximately 0.83% based on dry weight[23].

Method of Extraction of Manjistha

Dried manjistha root were obtained from a local market and roots were washed after washing the entire root they were dried for 3 to 4 days in shade. In a grinder, the roots were chopped into tiny pieces and ground into a fine powder. 500 ml of ethanol and water (1:1) were added to a round-bottom flask after 50 g of powder had been weighed, packaged in a muslin cloth bag, and placed within the Soxhlet extractor's body. The Soxhlet extractor, round-bottom flask, and condenser were then installed on the apparatus with the use of clamps and a stand. For a constant water flow, the condenser was fitted with a rubber tube that was connected to the tap water. The isomantle was used to heat the solvent, causing it to start evaporating and flow through the device and into the condenser. The cycle started when the solvent level reached the siphon and emptied back into the flask. The procedure was designed to take six hours to complete. Ultimately, the extract was filled the flask with a circular bottom. After the procedure was complete, the solvent was removed from the plant material (about 2-3 ml) in the glass bottom flask using a rotary evaporator set at 40°C. The extract was stored in a porcelain bowl until all of the ethanol was gone[24, 25].

Preparing Flaxseed Gel

- Water and flaxseed were mixed in a container.
- The mixture was heated for 3 to 4 minutes until it begins to boil, constantly stirring.
- Turn off the heat when a white, frothy gel-like substance forms.
- Allow the mixture to cool down for 20 to 30 minutes.
- Use a slight cotton fabric to strain out the gel from the flaxseed mixture[26].

Figure 2: Method of extraction of licorice and Manjistha extract through Soxhlet apparatus

Method of Extraction of almond oil

- Dried almonds are ground into a fine powder or paste using a mechanical grinder or mill.
- The ground almond paste is then subjected to pressure to extract the oil.
- Cold pressing is commonly used, where hydraulic presses or screw presses exert pressure on the almond paste, squeezing out the oil without applying heat. This helps preserve the oil's nutritional value and flavor[27].

Figure 3: Method of extraction of carrot seed oil and almond oil through Clevenger apparatus

Extraction of rose water

- Hydro distill 60g of dried rose petals with 1.5 liters of distilled water for 4 hours.
- Collect 800ml of rose water.
- Store the collected extract in an sealed container in the refrigerator at 4°C for further study[28].

COMPOSITION OF HERBAL FACIAL SERUM

Table 2: Working formula for serum

Ingredients	Standard formula for (100ml)	Working formula (30ml)
Liquorice Extract	12 ml	3 ml
Manjistha Extract	10 ml	2 ml
Carrot Seed Oil	12 ml	5 ml
Almond oil	8 ml	4 ml
Flax Seed Gel	12 ml	5 ml
Rosewater	18 ml	4 ml
Aloevera Gel	20 ml	4 ml
Glycerine	5 ml	2.5 ml
Salicylic Acid (2%Conc.)	3 ml	0.5 ml

Formulation of fairness serum

Procedure: Mechanical Homogenization method

A reasonable amount of oil and extracts were obtained, and mechanical homogenization was used to minimize size. The various aqueous extracts were thoroughly combined in an adequate amount, gradually added to the oil extracts in the homogenizer that had had their size decreased, and triturated until a thick, transparent liquid was created. Salicylic Acid was added as a preservative to the mixture. As a flavoring agent, an appropriate amount of rosewater was added[29]. The formula for serum is shown in the Table 2.

Figure 4: Final formulation of faces serum

EVALUATION PARAMETERS

Physical Evaluation: Visual observations were made on the formulation's color and look. The formulation shows uniform distribution of extracts. This test was confirmed by visual appearance and by touch.

pH Value: The calibration of a pH meter was done with a typical buffer solution. After correctly measuring and dissolving about 1 milliliter of the face serum in 50 milliliters of water, the pH of the solution was determined. Since the skin has an acidic spectrum, the skin serum's pH should be amid 4.1 and 6.7[30].

Determination of Spreadability: A surface was covered with two grams of serum sample. A pan had a slide fastened to it, and 20 grams of weight had been added. Spreadability was determined by measuring the amount of time (in seconds) needed to separate the upper slide from the surface[31].

- **Irritancy:** After applying the serum and noting the time, the area was observed for up to 24 hours to check for erythema, edema, and irritation before being reported. The formulation did not demonstrate any signs of irritation, erythema, or edema, according to the results[32].

- **After feel:** Following the serum's distribution among users and their application, users reported to us that the serum had a calming and pleasant impact, suggesting that it had an emollient and moisturizing function as well as being non-irritating and non-sensitive to the skin[33].

- **Globule size determination:** To verify the globule size, serum was examined under a microscope. Under a microscope, a drop of serum was diluted with water, placed on a glass slide, and covered with a glass cover[34].

Stability Studies

Formulation and development of a pharmaceutical product is not complete without proper stability analysis carried out on it to determine physical and chemical stability and thus safety of the product. The stability studies are accepted out as per ICH guiding principle. For a few months, a short-term accelerated stability study was conducted for the developed formulation. A 45-day short-term accelerated stability study was conducted for the formulation. The product were stored at different storage conditions of temperatures such as 3-5º C, 25º C RH=60% and 40ºC±2% RH=75%[35].

Cyclical Temperature Test

There is no set temperature or humidity throughout this test. Every day, the temperature was cycled in this test. At room temperature and frizzing temperature to stimulates the changes in temperature[36].

PHOTOCHEMICAL SCREENING OF SECONDARY METABOLITES

The preliminary phytochemical analysis of the plant extracts was performed using the standard protocol as describe by Khandelwal, and Kokate, to categorize the existence of phytoconstituents resembling flavonoids, glycosides and alkaloids etc. Based on the occurrence of phytoconstituents, the subsequent investigation of secondary metabolites was carried out:

Test for alkaloids

- **Dragendroff's test:** Take 2 ml of extract, little drops of reagent were added. A turbid orange/orange-red precipitate was observed in the existence of alkaloids

- **Hager's test:** A few drops of Wagner's reagent were added in 2–3 ml extract. Then, a reddish-brown precipitate was observed that confirms positive[37].

Test for Tannins

- **Ferric chloride reagent test:** 2–3 drops of 5% ferric chloride solution were taken and they are poured on both extracts. Then the formation of green/greenish-black colour shows the occurrence of tannins[38].

Test for Flavonoids

- **Lead acetate test:**10% lead acetate solution was mixed with the extracts, this forms a yellow colour precipitate which suggests the presence pf flavonoids[39].

Test for Phenolic Compounds: An equal amount of 1% Ferric chloride solution and 1% Potassium ferrocyanide was mixed; 3 drops of this prepared mixture were added to the 2ml of extracts. The positive outcome shows the development of a bluish colour.

Test for Glycosides

- **Keller-Killani Test:** Glacial acetic acid was poured into 2 ml. extract and one drop 5% $FeCl_3$ and conc. H2SO4. Reddish brown color l occurs at the intersection of the two liquid layers and the upper layer of bluish green indicates the occurrence of glycosides[40].

- **Glycosides test:** in small portion of extract add few ml of water were added and shaken effectively and few ml of aqueous solution of NaOH was poured. The emergence of yellow colour shows the existence of glycosides.

- **Concentrate H2SO4 Test:** Few amount of extract were taken and 2ml. glacial acetic acid, 5% FeCl3 and conc. H2SO4 were mixed, the emergence of brown ring illustrates the occurrence of glycosides.

- **Molisch's Test:** few drops of regent were mixed with 1ml of extract, and 2 ml of concentrate H2SO4 was added cautiously with solution. Development of violet ring at the intersection indicates the existence of glycosides[40].

RESULT AND DISCUSSION

The formulation was evaluated for the following parameters:

Organoleptic Evaluation

Various physical parameters such as color, odor, texture, pH, and appearance of serum were evaluated, and the results were summarized in Table 3.

Physical appearance: Serum formulation was reddish brown viscous liquid preparation with a smooth homogeneous texture and glossy appearance. Consistency was established to be good. It is shown in Figure 4.

Table 3: Organoleptic evaluation of formulated face serum

S.NO.	Parameters	Results
1.	Colour	Reddish – Brown
2.	Odor	Floral
3.	Texture	Smooth homogenous
4.	Appearance	Translucent
5.	pH	5.1

Physical evaluation: The formulation was re-dispersed within seconds after doing the redispersion test. The percentage spread by area was found to be 70.8%. The viscosity was in the range of 2300cps.

Table 4: Preliminary phytochemical screening

Phytochemical screening of formulation revealed that the extract contain Alkaloids, Proteins, Carbohydrates, Tannins, and Glycosides.

S.no.	**Test Performed**	**Result**
1.	Spreadability test	Good
2.	Irritancy test	Non-irritant
3.	Dragondroff's test	Positive
4.	Hagner test	Positive
5.	Ferric Chloride reagent test	Positive
6.	Lead Acetate test	Negative
7.	Molish test	Positive
8.	Conc. H2SO4	Positive
9.	Keller-Killani test	Positive
10.	Glycosides test	Negative

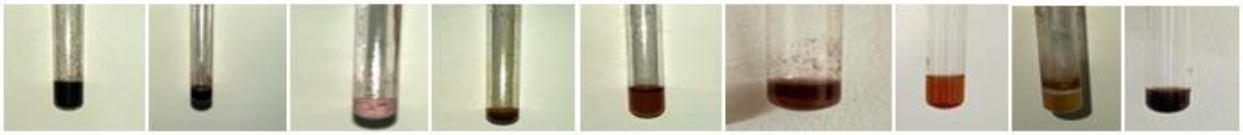

Figure 5: Preliminary phytochemical screening of formulation

Figure 6: Showing spreadability of face serum and globule size determination through microscope

Table 5: Stability studies of the formulation

Temperature	Evaluation parameters	Observation in Days			
		0	**15**	**30**	**45**
3 – 5° C	Visual appearance	Reddish – Brown	Reddish – Brown	Reddish – Brown	Reddish – Brown
	Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
Room temp (25°C RH=60%)	Visual appearance	Reddish – Brown	Reddish – Brown	Reddish – Brown	Reddish – Brown
	Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
40°C±2% RH=75%.	Visual appearance	Reddish – Brown	Reddish – Brown	Reddish – Brown	Reddish – Brown
	Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good

CONCLUSION

The aim of the study is to formulate and assess of an anti-aging herbal face serum containing liquorice, Manjistha, carrot seed oil, almond oil, Aloe Vera, rose water, and glycerin provide a promising solution for addressing various skin concerns associated with aging. Liquorice, known for its anti-inflammatory and skin brightening properties, contributes to reducing hyperpigmentation and evening out skin tone. Manjistha complements liquorice by aiding in detoxification and promoting skin rejuvenation, helping to diminish the appearance of fine lines and wrinkles. The inclusion of carrot seed oil, rich in antioxidants and vitamins, offers protection against free radicals and environmental stressors, promoting skin elasticity and firmness. Almond oil, renowned for its moisturizing and emollient properties, deeply nourishes the skin, leaving it supple and hydrated. Aloe Vera, with its soothing and healing abilities, helps to calm irritated skin and improve skin texture. Rose water acts as a natural toner, balancing the skin's pH and imparting a refreshing feel, while glycerin acts as a humectant, attracting moisture to the skin and maintaining its hydration levels. Through rigorous evaluation, including stability testing, sensory evaluation, and efficacy studies, this

herbal face serum has demonstrated promising results in combating signs of aging. The spreadability was found to be good. No residues were formed and were easy to wash out. Its lightweight texture and fast-absorbing formula make it suitable for all skin types, providing long-lasting hydration and a youthful glow. With consistent use, this anti-aging herbal face serum offers a holistic approach to skincare, addressing multiple concerns while promoting overall skin health and vitality.

CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation**.**

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