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DEVELOPMENT OF A NATURAL HERBAL SERUM USING MANGO SEED EXTRACT AND ITS EVALUATION FOR SKIN BRIGHTENING ACTIVITY– A RESEARCH

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ABSTRACT

The specific objective of the present study was to develop and evaluate a herbal face serum based on mango seed extract, which could be effective as a skin brightener, antioxidant, and anti-aging agent. Due to the growing demand for natural, safe, and effective cosmetic products, mango seed kernel, an agro-waste product of Mangifera indica, was chosen as the bioactive agent of choice because of its rich content of polyphenolic, flavonoid, tannoid, and fatty acid constituents, which are reported to possess antioxidant properties beneficial for skin care. Literature survey confirmed the antioxidant, antimicrobial, and anti-inflammatory properties of mango seed kernel extract, making it a good choice as a cosmetic agent.

The extract was prepared by employing the Soxhlet method of extraction, and the oil-in-water emulsion-based herbal face serum was formulated by using various excipients such as jojoba oil, olive oil, glycerine, tween, xanthan gum, and phenoxyethanol. A total of four formulations (F1-F4) of the herbal face serum were developed, and they were evaluated for various physicochemical parameters, including organoleptic properties, pH, viscosity, homogeneity, and spreadability of the formulations. Phytochemical analysis of the extract confirmed the presence of bioactive constituents, including alkaloids, flavonoids, tannins, glycosides, and proteins.

The formulations showed good physical properties in terms of consistency, stability, and skin compatibility. Antioxidant activity was performed by employing DPPH and FRAP assays, showing significant free radical scavenging activity by the optimized formulation, comparable to standard antioxidant agents. The antimicrobial study of the formulation against Staphylococcus aureus showed effective inhibition of the microorganism, indicating its potential in preventing acne and skin infections caused by the microbe.

In conclusion, the formulated herbal face serum using mango seed kernel extract was found to be stable, effective, and suitable for topical use. The importance of using natural, eco-friendly, and cost-effective ingredients in cosmetic formulations has been emphasized by the results of the study. The trend of using herbal products in skincare products has gained importance in recent times, and the results of the study show the potential of using mango seed kernel as a value-added product in skincare products as a skin brightener or anti-aging agent.

INTRODUCTION

SKIN

Skin, also known as the integumentary system, is the largest organ in our body, covering the entire external surface, measuring up to $2m^{2,}$ and weighing approximately 4.5−5kg in an average adult. Skin is the first physical barrier protecting us from the external environment. Skin is made of three layers: the outermost layer, the epidermis, the structure below it, the dermis, and the structure below the dermis, known as the subcutaneous tissue. The anatomy, structure, and composition of each of these layers vary, depending on the function and role of that layer

Epidermis

The epidermis has approximately 0.5−1.5mm thickness and is avascular, meaning it doesn't have any blood vessels, and is composed of keratinised, stratified squamous cells. The skin areas on the palms, soles, and fingertips are known as the 'thick skin,' and the skin on other areas of the body is known as the 'thin skin. In thin skin, the epidermis is composed of four epithelial layers: the stratum corneum, stratum granulosum, stratum spinosum or prickle cell layer; and stratum germinativum or stratum basale. In thick skin, the epidermis is composed of five epithelial layers, including the stratum corneum; stratum lucidum; stratum granulosum; stratum spinosum or prickle cell layer; and stratum germinativum or stratum basale.

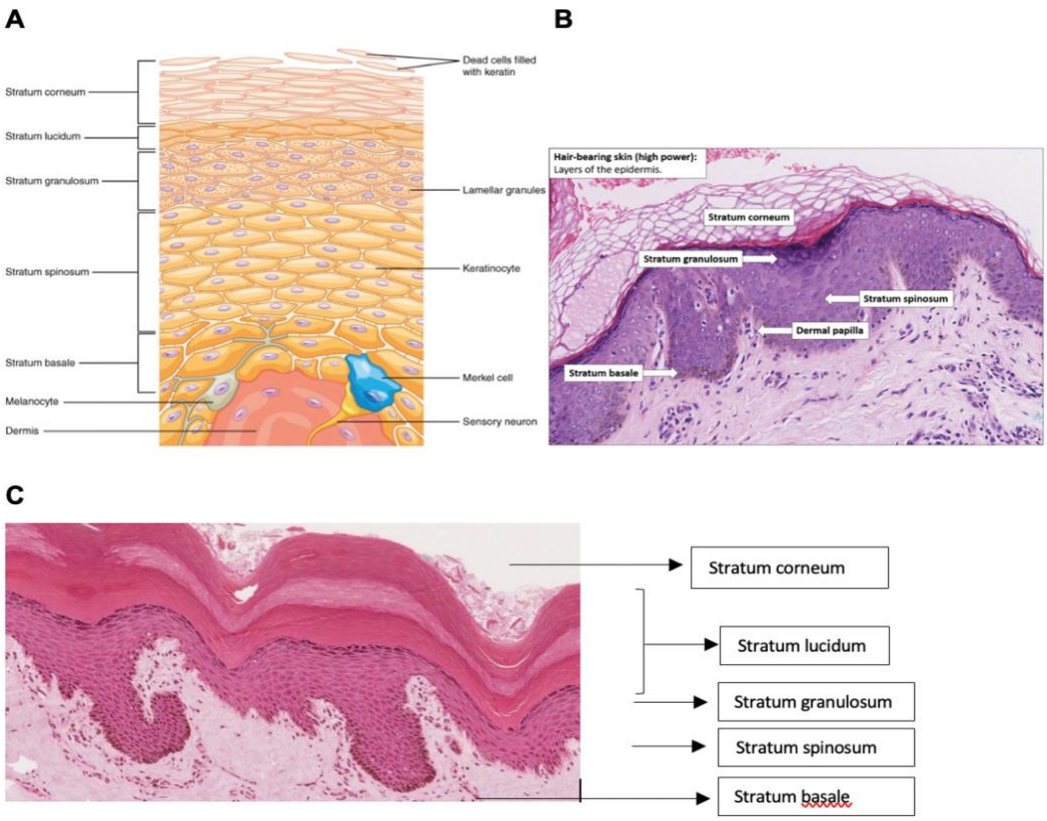


Figure 1: (a) Schematic presentation of the epidermis layer of the skin. (b) Histology image of skin. (c) Histology of skin with stratum lucidum.

Dermis

The dermis is considered the "core" of the integumentary system (dermis = skin), as distinct from the epidermis (epi-= upon or over) and hypodermis (hypo-= below).

The dermis is the thicker structure containing vessels and nerves, nourishing and supporting the epidermis. The dermis itself is divided into two regions: the superficial papillary region and the deep reticular region. The superficial papillary region is the thinner upper layer, composed of loose connective tissue that contacts the epidermis connective tissue. It consists of the extracellular matrix and fibroblasts. Fibroblasts also secrete fibronectin and hyaluronic acid, two major components of the extracellular matrix, which play a role in wound healing. The superficial dermis also contains blood vessels, lymphatics, epithelial cells, small muscles, and neurons.

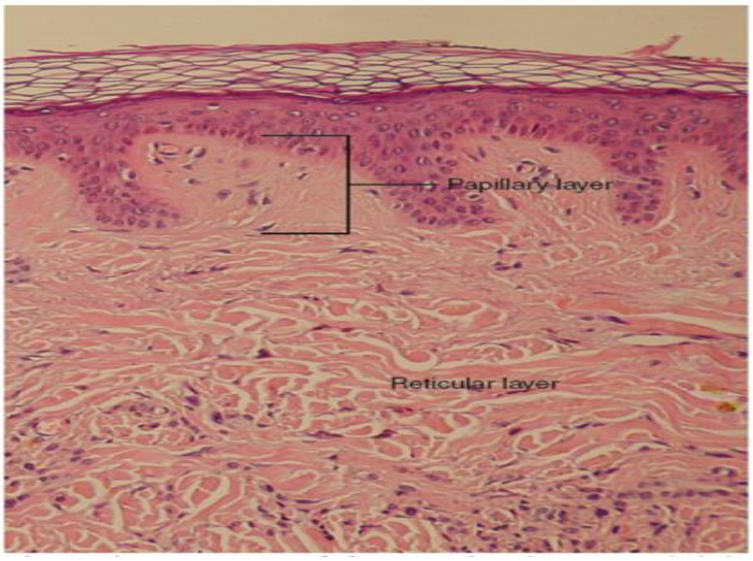


Figure 2: Histology of the skin showing the different sections of the dermis: the papillary layer and the reticular layer.

Hypodermis

This layer, also known as the subcutaneous layer or superficial fascia, lies beneath the dermis and anchors the dermis to the underlying fascia surrounding muscle or bone. The hypodermis is mainly composed of adipose tissue, with the role of fat storage, and provides cushioning and insulation. The hypodermis also contains well-vascularised, loose, and areolar connective tissue.

Cell types in the skin

Keratinocytes, Langerhans' cells, Melanocytes, Merkel cells

FUNCTION OF THE SKIN

- Protection
- Regulation of body temperature
- Immunological function
- Endocrine function
- Excretory function
- Regulation of water electrolyte balance
- Sensory function
- Blood reservoir
- Drug delivery route.

TYPES OF SKIN

- Dry Skin
- Normal Skin
- Oily Skin
- Combination Skin
- Sensitive Skin

Dry Skin

This type of skin is often flaky, always has some sort of peeling and can even have a powdery look. It lacks hydration and feels tight on your face. Dry skin also leads to sensitivity to 5 products.

Normal Skin

This type of skin is neither too oily nor too dry. It is generally less reactive to external threats and easier to maintain.

Oily Skin

It is characterized by its greasiness throughout the face. This happens because of the overactivity of sebaceous glands, leading to excessive sebum production on the skin.

Combination Skin

A combination of skin is identified as having an oily T zone and dry cheeks. The oiliness and dryness are a response to weather conditions, with summers causing extreme oiliness and winters causing dryness.

Sensitive Skin

It is characterized by its reactions to products, foods and environmental factors in a manner that is more persistent with constant triggers.

FACE SERUM

Skin Serum is a skincare product that is applied on skin after washing but before moisturising with the goal of quickly delivering potent nutrients to the skin. Skin care treatments called serums are made to deliver concentrated amounts of specific active ingredients to the skin. There are numerous unique varieties of serums available that perform special tasks, from hydration to skin lightening. The serum is especially well-suited for this endeavour since it is

composed of tiny molecules that may permeate the skin deeply and provide an extremely high awareness of active components. This makes them an exceptional tool for concentrating on skincare issues, such as pigmentation and ageing signs.[1]

Serum is defined as a light, easily absorbed oil or water-based liquid that spreads on the skin. It gets rapidly absorbed and easily penetrates the deeper layers of the skin. Also has a non-greasy finish and an intensive formula that contains a high concentration of active substances. It contains skin-smoothing ingredients that enhance skin texture and make skin soft, silky smooth, and fair. The formulation has good spread ability and making the pores appear smaller and increasing the moisture level. Face serum delivers the active ingredient into the skin and removes the use of hazardous chemicals in giving instant results.

Advantages of face serum

- ✓ Soothes irritated skin
- ✓ Absorbs quickly into the skin
- ✓ Improves the appearance of fine lines and wrinkles
- ✓ Protects the skin from free radicals and future damage
- ✓ Has the potential to provide more visible results
- ✓ Feels light on the skin

Disadvantages of Face Serum

- ✓ Not effective for all skin issues
- ✓ Can be pricey
- ✓ Difficult to select the proper one.
- ✓ If not used effectively, it risks being wasted.

GENERAL FORMULATION OF FACE SERUM

The formulation of face serum involves carefully selecting and combining ingredients to create a product that addresses specific skincare concerns and delivers desired benefits. Here's a basic outline of the formulation process.

- Active ingredient selection: Identify key active ingredients based on their skin care benefits. These could include botanical extract, vitamins, and other compounds known for their moisturizing, anti-aging, brightening, or soothing properties. The formulation of a

facial serum involves carefully selecting and combining ingredients to create a product that addresses specific skincare concerns and delivers desired benefits.

- Base Ingredients: Choose a base or carrier for the serum, such as water, glycerine, or a combination of water and glycerine. This forms the bulk of the product and helps deliver the active ingredients to the skin.

- Emulsifiers and Stabilizers: Add emulsifiers and stabilizers to ensure that the ingredients blend smoothly and maintain their stability over time.

- Preservatives: Incorporate preservatives to prevent microbial growth and extend the shelf life of the serum. Examples of preservatives include phenoxyethanol.

- Fragrance and Colour: Optionally, add fragrance or natural essential oils for scent, and colorants for aesthetic appeal. Keep in mind that fragrance can be sensitizing for some individuals, so consider using minimal amounts or opting for fragrance-free formulations.[2]

METHODOLOGY

FORMULATION OF HERBAL FACE SERUM

➢ **Collection of Material**

Ripe mango seeds were collected from the local market. The seeds were washed with tap water to remove their impurities.

➢ **Preparation of Sample**

Mango seeds were washed and then split as the first step in sample preparation, and the covering layer of the core was removed to obtain the maximum kernel portion. The kernels were then chopped, dried, and made into flour to obtain a small particle size for better solvent penetration. Kernel flour was packaged and placed in the freezer to await the extraction process.

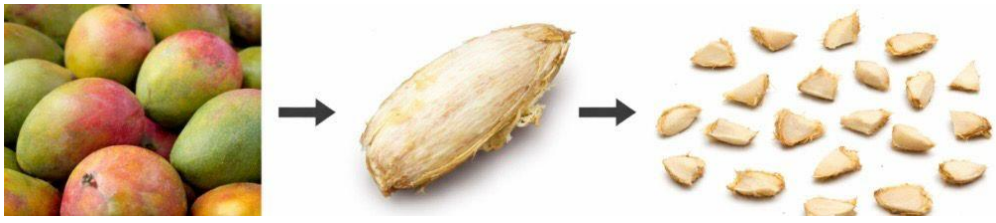


Fig. 3: Step in the preparation of mango seed kernel powder.

➢ **Extraction procedure**

Soxhlet extractor: A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz Von Soxhlet. The Soxhlet extractor is divided into three parts.

1) Boiling flask 2) Thimble 3) Condenser

Fig. no. 4: Soxhlet apparatus.

Fig. no. 5: Distillation process.

The extraction process uses a Soxhlet apparatus. For each experimental unit, 20 g of mango kernel flour was used, wrapped in cotton and filter paper, and then placed in a Soxhlet sleeve. This sleeve was inserted into the Soxhlet thimble facing upwards. The solvent was put into the thimble until it was full and flowed into a 3-neck flask of 300 mL that was under the thimble. The solvent continued to be added until it covered the Soxhlet sleeve. The reflux condenser, which has been connected to a water pump, was then connected to the Soxhlet thimble. The flask was heated using a heating mantle at 80℃ and adjusted using a thermometer setting. The extraction process continues until the solvent in the thimble becomes clear. The sleeve was removed from the thimble, and the mango seed kernel and solvent were separated by heating the flask until the solvent evaporated and separated from the thimble. The mango seed kernel product in the flask was transferred to a dark packaging container and stored in the freezer.

METHOD OF PREPARATION OF A HERBAL FACE SERUM[2]

- **Preparation of the oil phase**

  The oil-soluble ingredients, such as jojoba oil and olive oil, are taken into one beaker and melted at 70°c

- **Preparation of the water phase**

  The water phase is prepared simultaneously by mixing mango seed extract with phenoxyethanol in another beaker.

- **Preparation of emulsion**

Emulsion was prepared by adding the oil phase into the water phase dropwise under mechanical stirring at 700 to 800 rpm to obtain an O/W biphasic emulsion.[4]

Fig no. 6: Mango seed kernel face serum.

FORMULATION DESIGN OF HERBAL FACE SERUM

Four formulations were prepared, and the formulation design is as shown below.

Table no 1 – List of Quantities of Materials.

SL. NO.	INGREDIENTS	F1	F2	F3	F4
1	Mango seed kernel extract (ml)	2	2	2	2
2	Jojoba oil (ml)	2	2.5	3	3.5
3	Olive oil (ml)	1	1	1 .5	1
4	Glycerine (ml)	2	2	2	2
5	Tween 20(ml)	0.5	0.5	-	-
6	Tween 80 (ml)	-	-	0.5	0.5
7	Xanthan gum(g)	0.3	0.3	0.3	0.3
8	Phenoxy ethanol (ml)	0.2	0.2	0.2	0.2
9	Distilled water (30ml)	q.s.	q.s.	q.s.	q.s.

Drug-excipient profile

Drug profile: Description of drugs taken for the formulation and evaluation of mango seed extract-based herbal face serum for skin brightening activity.

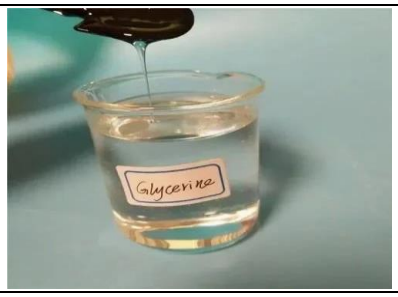
Table no 2– List of drug profiles.

DRUGS	MANGO SEED KERNEL	JOJOBA OIL	OLIVE OIL
Synonym	Aam Guthli, Aam Magaj, Pit, Core	jojoba wax	Common Olive Oil, Extra Virgin Olive Oil, Feuille d'Olivier, Huile d'Olive, Refined Olive Oil, Virgin Olive Oil
Biological source	The biological source of the mango seed kernel is the large, fibrous seed found inside the mango fruit, from the tree *Mangifera indica*	Jojoba oil is derived from the seed of the jojoba plant, Simmondsia chinensis, belonging to the family Simmondsiaceae.	Olive oil is derived from the ripe fruit of the olive tree, specifically Olea europaea, belonging to the family Oleaceae.
Chemical constituents	carbohydrates, fats, proteins, fiber, and minerals, with significant amounts of starch, polyphenols (antioxidants), and fatty acids	eicosenoic acid (gadoleic acid), which makes up over 70% of the fatty acid content, and erucic acid and oleic acid	Triacylglycerols, Fatty Acids (Monounsaturated Fatty Acids, Polyunsaturated Fatty Acids, Saturated Fatty Acids), Vitamins A, D, and Polyphenols
Uses	moisturizing, anti-aging, and soothing properties	soothe dry skin and promote soft, healthy-looking skin, help prevent the skin from flaking.	moisturizing skin and hair, acting as a gentle makeup remover, and soothing minor sunburns
Picture			

Excipient Profile: Description of excipients used in the preparation shown in the 2

Table no 3 – List of excipient Profile.

EXCIPIENTS	TWEEN 20	GLYCERINE	XANTHAN GUM
Synonym	Polysorbate 20, polyoxymethylene sorbitan monolaurate	Glycerol	Corn sugar gum, polysaccharide B 1459
Biological source	Tween 20 is derived from biological sources like coconut oil and corn/wheat, belonging to the polysorbate chemical family	Simple polyol compound that can be derived from both natural and synthetic sources	Xanthan gum is derived from the bacterium Xanthomonas campestris, which belongs to the family Pseudomonadaceae
Functional	emulsifier, detergent,	Humectant, emollient,	thickener, stabilizer, and

category	wetting agent, and solubilizer	preservative, viscosity modifier.	emulsifier
Description	Colour: clear, yellow to yellow-green viscous liquid Odour: mild odour Melting point: -14°C to -64°C Boiling point:>100°C	Colour: clear colourless liquid Odour: odourless Melting point: 17.8 °C Boiling point: 290 °C	Colour: off-white to pale yellow Odour: odourless or nearly odourless powder Melting point: around 64.43°C
Storage	tightly sealed in a cool, dry, well-ventilated place, protected from light, usually at room temperature,	Stored in a cool, dry place.	Stored in an airtight container in a cool, dry place
Uses	Solubilizer, emulsifier, surfactant/detergent, enhancing texture	Humectant, moisture to the skin, improving hydration	thickener, stabilizer, and emulsifier to improve product texture, consistency, and shelf life
Picture			

EVALUATION

Phytochemical screening

Freshly prepared seed kernel extracts were tested for the presence of phytochemical constituents using standard methods.[5]

Preformulation studies

Preformulation testing is the first step in the rational development of the dosage form of the drug substance. The overall objective of the study is to generate information that is useful in developing stable dosage forms. The preformulation studies were carried out.

Organoleptic evaluation

The formulations were characterized for organoleptic properties such as colour and odour. The formulations are visually inspected for their clarity and the presence of any foreign particles.

Homogeneity

The formulation was tested for homogeneity by visual inspection and touch.

Determination of pH

A pH meter was calibrated using a standard buffer solution. Nearly 1 ml of face serum was properly weighed and dissolved in 50 ml of distilled water, and finally, its pH was calculated. The skin has an acidic range, and the pH of the skin serum should be in the range of 4.1 -6.7.

Determination of spreadability

The spreadability was expressed in terms of time in seconds taken by two slides to slip off from the gel, placed in between the slides, under a certain load. The lesser the time taken for the separation of the two slides, the better the spread ability. Two sets of glass slides of standard dimensions were taken. 0.2g of the sample formulation was placed on one of the slides. The other slides were placed on the top of the gel, such that the serum was sandwiched between the two slides. 100gm weight was placed upon the upper slides so that the serum between the two slides was pressed uniformly to form a thin layer. The weight was removed, and the excess serum adhering to the slides was scraped off. The time taken for the upper slide to travel the distance of 6.0cm and separate from the lower side under the influence of the weight was noted. Spreadability was calculated by using the following formula

S= [ML/T]

Determination of Anti-microbial Activity

Cup-plate Method

The inoculum S. aureus culture is prepared in the nutrient agar broth medium. Placed a sterile disc saturated with the formulated face serum and a marketed one aseptically by using forceps in the petri dish. The disc was allowed to diffuse, and after some time, the plates were incubated at 370 °C for 24 hours. After 24 hours, the petri dishes were observed for ZOI, and the diameter of the zone of inhibition was measured in millimeters.

In vitro antioxidant activity

Determination of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

Ethanol-DPPH solution (0.1 mM DPPH in ethanol) was prepared fresh. The DPPH solution (2.9 mL) was placed in a glass cuvette, and the absorbance at 515 mm in time t = 0 min (to) was measured. 0.1 mL of the sample extract was added, and the mixture was shaken vigorously and kept in the dark at room temperature for 30 min (t3o). The absorbance at 515 mm was then measured using a Spectro quant Pharo 100 spectrophotometer (Merck, United

States). A standard curve using Trolox was prepared, and the percentage reduction of DPPH was calculated as

% DPPH reduction = [Absorbance (t 3o)/ Absorbance (to)] × 100

Ferric reducing antioxidant power (FRAP) assay

This assay is based on the antioxidant capacity to convert ferric ions into ferrous ions. Once the reduction of ferric ion occurs, a blue colour is developed.

Preparation of phosphate buffer (pH 6.6, 0.2M)

Dissolve 27.60g of sodium dihydrogen phosphate monohydrate in 900ml of water. Adjust to pH 6.6 with 10M sodium hydroxide and dilute with water to produce 1000ml.

Procedure

Stock solutions of facewash formulations were prepared, and this stock solution was serially diluted separately to obtain different concentrations.

To the 1ml of sample solution, add 2.5ml phosphate buffer (6.6Ph, 0.2 M), mix well, then add 2.5ml of 1% ferrocyanide solution. Cover with aluminium foil and incubate at 50˚C for 20 minutes in a water bath. Shake the reaction mixture and add 2.5ml of 10% trichloroacetic acid. After thorough mixing, take 2.5ml from the reaction mixture and add 2.5ml of distilled water, followed by adding 0.5ml of 0.1% of ferric chloride. A bluish colour is developed, and absorbance is measured at 593 nm. Ascorbic acid is used as the standard, and distilled water as the blank.

RESULT AND DISCUSSION

Phytochemical screening

Table no 4. List of phytochemical constituents.

Sl.NO	TEST FOR	INFERENCE
1.	Alkaloids	**Presence**
2.	Flavonoid	**Presence**
3.	Tannins	**Presence**
4.	Glycosides	**Presence**
5.	Carbohydrates	**Presence**
6.	Protein	**Presence**
7.	Steroids	**Presence**

Preparation of herbal face serum

Four formulations of herbal face serum, F1, F2, F3, and F4, were prepared.

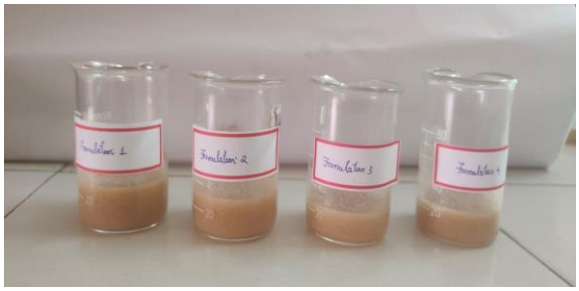


Fig. 7: Preparation of a herbal face serum.

All the prepared formulations were subjected to the following evaluation parameters.

EVALUATION

ORGANOLEPTIC EVALUATION

The results of organoleptic evaluations are shown in the Table.

Table no 5. Organoleptic evaluation.

Formulations	Colour	Odour	Consistency
F1	Light brown	Characteristic	Gel
F2	Light brown	Characteristic	Gel
F3	Light brown	Characteristic	Gel
F4	Light brown	Characteristic	Gel

The formulations F1, F2, F3, and F4 were observed as light brownish in colour.

pH

The pH of all four formulations was recorded by a digital pH meter

Fig 8. pH meter.

Table 6: Evaluation of Ph.

Formulations	pH
F1	1.30
F2	2.45
F3	2.96
F4	4.20

VISCOSITY

The Brookfield viscometer was used to measure the viscosity of each of the four herbal face serum formulations.

Fig. 9: Brookfield viscometer.

Table 7: Evaluation of viscosity.

Formulations	Viscosity (cps)
F1	1500
F2	1450
F3	1100
F4	1300

The viscosity of all the prepared formulations was found to be in the range of 1100- 5600 cps at 100 rpm.

4.3.4. SPREADABILITY

Spread ability plays a considerable role in patient compliance and ensures uniform application of the herbal face serum. The values of spreadability indicate that the face serum is easily spreadable by a small amount of shear.

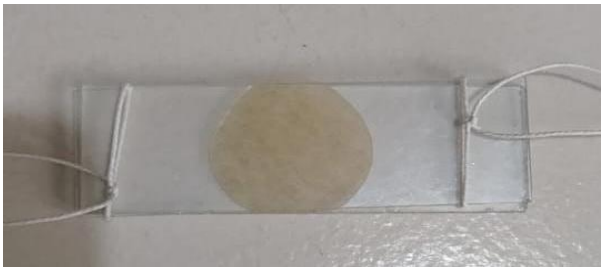


Fig no. 10: Spreadability of formulation.

Table no. 8: Spreadability studies.

Formulations	Spread ability(mm^2)
F1	18
F2	24
F3	32
F4	36

Spreadability of the formulations is shown in Table 8. Formulations are spreadable, and F1 has a higher spreadability.

ANTIMICROBIAL ACTIVITY

Optimized formulation F4 was selected and subjected to microbial study by using the cup plate method, and the zone of inhibition was determined.

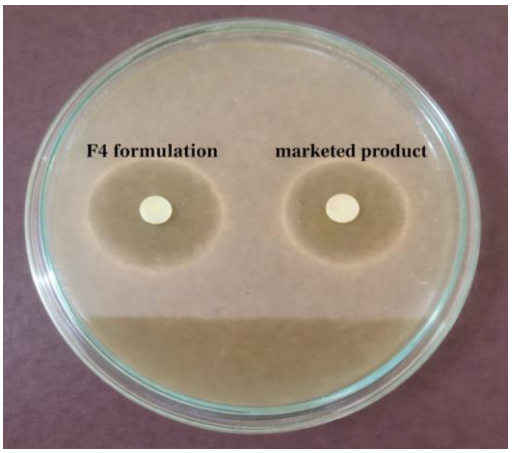


Fig no. 11: Zone of inhibition.

Table 9: Zone of inhibition.

Formulation	Zone of inhibition (mm)
F4	15
Minimalist face serum	13

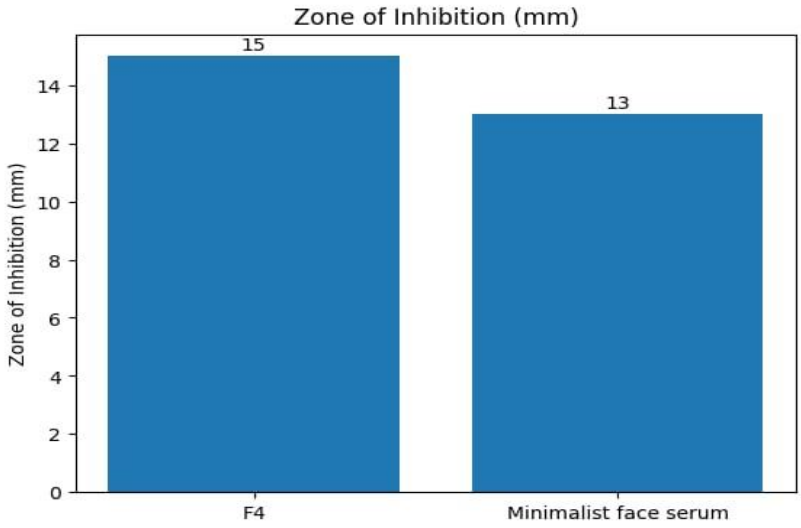


Fig no. 12: Graph of antimicrobial activity.

The antimicrobial test demonstrated significant activity against acne-causing bacteria (Staphylococcus aureus) and found that the zone of inhibition for the formulation F4 is close

to that of the marketed formulation. The zone of inhibition for the formulation was found to be 13 mm, which is almost close to the zone of inhibition of the standard formulation.

IN VITRO ANTIOXIDANT ACTIVITY

DPPH ASSAY

Table 10: DPPH scavenging activity of formulation.

Formulation concentration (µg/ml)	Percentage inhibition (%)
F1	75.885
F2	76.18
F3	75.81
F4	81.51

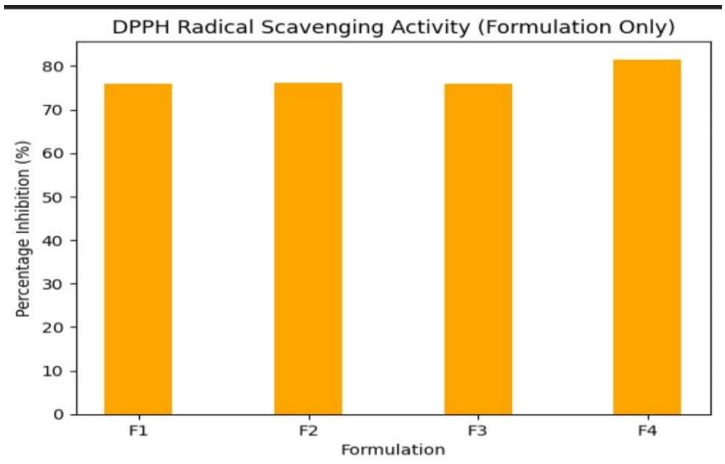


Fig. 13.Graph of DPPH Assay.

Antioxidant assay by DPPH radical scavenging activity was carried out for the standard (ascorbic acid) and all 5 formulations. Percentage inhibition was calculated, and the result indicates F4 have highest antioxidant activity among all the formulations. F4 has antioxidant activity closer to the standard. Hence, F4 shows antioxidant properties.

FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

Fig. 14: FRAP assay of standard.

Table 11: FRAP assay of standard.

Standard Concentration(µg/ml)	Percentage Inhibition (%)
20	69.15
40	73.25
60	78.72
80	83.02
100	97.26

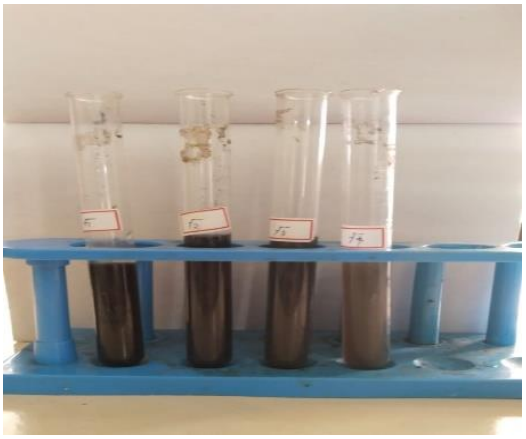


Fig. 15: FRAP assay of formulation.

Table 12: FRAP assay of formulations.

Formulation concentration (10 µg/ml)	Percentage inhibition (%)
F1	85.57
F2	90.32
F3	97.58
F4	99.84

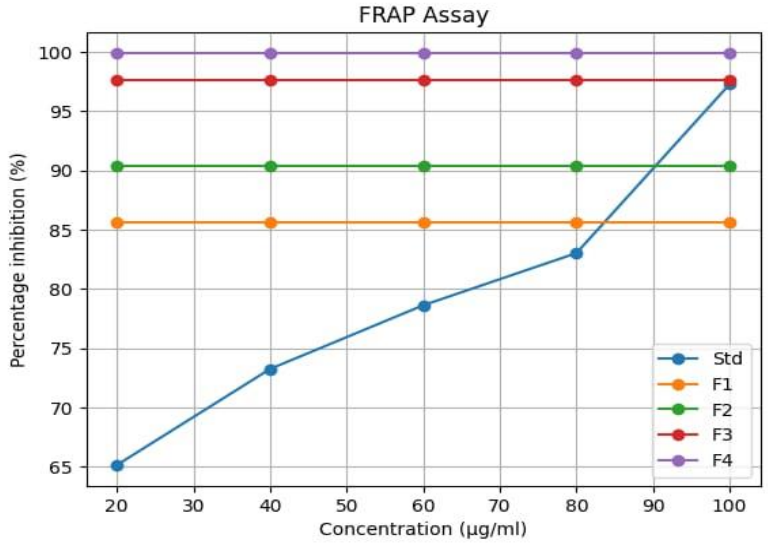


Fig. 16: Graph of FRAP assay.

FRAP assay was carried out for all the formulations, standard, and the percentage inhibition was calculated. From the result, the F4 formulation has a greater percentage of inhibition. Hence, it shows significant antioxidant activity.

SUMMARY AND CONCLUSION

The present study focused on the formulation and evaluation of a herbal face serum containing mango seed kernel extract for skin brightening and antioxidant activity. Mango seed kernel, an agro-waste obtained from *Mangifera indica*, was selected as the active ingredient due to its rich content of polyphenols, flavonoids, and fatty acids, which are known for their antioxidant, moisturizing, and skin-protective properties. The extract was successfully obtained using the Soxhlet extraction method and incorporated into an oil-in-water emulsion-based serum.

Different formulations (F1–F4) were prepared using suitable excipients such as jojoba oil, olive oil, glycerine, Tween, xanthan gum, and phenoxyethanol to improve stability, spread ability, and skin compatibility. The prepared formulations were evaluated for various physicochemical parameters, including organoleptic characteristics, homogeneity, pH, viscosity, and stability. The results indicated that the formulations were smooth, homogenous, and showed acceptable pH values within the skin-friendly range. The serum exhibited good spreadability, non-greasy texture, and rapid absorption, making it suitable for topical application.

The antioxidant and antimicrobial studies confirmed the potential of mango seed kernel extract in protecting the skin from oxidative stress and microbial contamination, which are major factors responsible for skin aging and pigmentation. Among the prepared formulations, the optimized formulation showed better stability, consistency, and effectiveness when compared with the marketed product.

Thus, the study concluded that mango seed kernel extract can be effectively utilized in the development of herbal cosmetic formulations. The formulated face serum is safe, economical, and environmentally sustainable as it utilizes natural and waste-derived resources. This work highlights the importance of herbal and plant-based ingredients in modern skincare and supports the growing demand for natural cosmetic products.

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