

FORMULATION AND OPTIMIZATION HERBAL NANOEMULSION BASED FACE SERUM

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Abstract : The present study aimed to formulate and optimize herbal nano-emulsion based face serum for improved skin delivery and therapeutic effectiveness. The both herbal extract was evaluated for anti-microbial activity and show satisfactory results. Different formulations were prepared using herbal extracts, oil phase, surfactant, and co-surfactant. The prepared nanoemulsions were evaluated for pH, viscosity, spreadability, globule size, polydispersity index. Optimization was carried out using factorial design to obtain a stable formulation with desirable characteristics. The optimized formulation showed good stability, nanosized globules, satisfactory spreadability, and significant antimicrobial activity. The study concluded that herbal nanoemulsion-based face serum is an effective and promising topical delivery system for cosmetic and skin care application.

IndexTerms - Herbal nanoemulsion, Face serum, Optimization, Factorial design, Globule size, Antimicrobial activity, Herbal cosmetics, etc.

Introduction:

Skin is the largest organ of the human body and serves as a protective barrier against external environment [1]. However, due to increasing pollutions, stress, hormonal imbalance and microbial contamination, various skin disorder such as acne, dermatitis, hyperpigmentation, premature aging and infections have become prevalent [2]. Conventional topical treatments, although effective, often produce undesirable side effects like irritation, dryness, allergic reactions and microbial resistance [2]. This has led to a growing interest in herbal based formulations, which are safer, cost effective and rich in biologically active phytoconstituents with therapeutic potential [3,4].

In recent years, nanoemulsion technology has gained significant attention as an thermodynamically or kinetically stable colloidal dispersions consisting of oil, water, surfactants and co-surfactant ,with droplet sizes typically ranging between 20-200nm [5]. Due to their small droplet size and large surface area, nanoemulsions enhance drug solubilization, permeability, stability and bioavailability of active compounds [5, 6]. Additionally, nanoemulsions improves skin hydration, ensure uniform distribution of active ingredients and facilitate deeper penetration into the epidermal layers [6]. These properties make nanoemulsions particularly suitable for formulating face serums, which require rapid absorption, non-greasy texture and high efficacy [7].

Herbal medicine such as tea tree oil, *Matricaria chamillae* (chamomile), *Lavandula angustifolia* (lavender) oil, *Butea monosperma* and vitamin E are known for their dermatological benefits. Tea tree oil (*Melaleuca alternifolia*) exhibits strong antimicrobial and anti-acne properties [8]. Chamomile possesses anti-inflammatory, soothing effects and inflammation, which help in reducing skin irritation [9]. Lavender oil contributes to wound healing and skin regeneration [10]. *Butea monosperma* is reported to have anti-inflammatory, antimicrobial and antioxidant properties beneficial for various skin conditions [11]. Vitamin E acts as a potent antioxidant protecting the skin from oxidative stress and premature aging [12]. The natural actives can be effectively incorporated into nanoemulsion-based serum formulations to enhance their stability, penetration and therapeutic potential [5,6].

Formulation development requires systematic optimization to achieve desirable characteristics such as stability, droplet size, viscosity and drug release. Factorial design is a statistical experimental design technique that allows simultaneous evaluation of multiple formulation variables and their interactions[13]. It helps in identifying the critical factors affecting performance and reduces the number of experimental trials while improving formulation efficiency [13,14].

In this study, a herbal nanoemulsion- based face serum is developed and evaluated for its effectiveness in managing various skin disorders. Additionally, factorial design is applied to optimize formulation parameters such as surfactant concentration, oil phase ratio and homogenization conditions to obtain a stable and effective product [14].

Advantages:

1. Enhanced skin penetration of herbal actives.
2. Better stability and bioavailability of formulation.
3. Non-greasy and easily absorbable preparation.
4. Improves antimicrobial and antioxidant activity.

Material and Method:

Pharmacognostic Investigation:

A. Plant Material:

The selected plant materials were collected from a local herbal garden/market and authenticated by a qualified botanist[18]. The collected materials were washed properly to remove dust and foreign particles and dried under shade at room temperature. The dried plant materials were powdered separately using a mechanical grinder and stored in airtight containers for further studies [18].

B. Plant collection and Authentication of plant material:

The medicinal plants selected for the present study were collected from local herbal gardens, agricultural fields or nearby markets during their appropriate season of availability [18]. Healthy, disease-free and mature plant materials were selected to ensure quality and authenticity of the crude drug [19]. The collected plant materials were washed thoroughly with distilled water to remove dust, soil particles and other extraneous matter. The cleaned materials were shade dried at room temperature to preserve the active phytoconstituents and prevent degradation due to direct sunlight exposure [20]. After complete drying, the plant materials were coarsely powdered using a mechanical grinder and stored in airtight containers for further pharmacognostic and phytochemical investigations [18]. Authentication of the collected plant material was carried out by a qualified taxonomist, botanist or pharmacognosist from a recognized institute or department [19]. The plant specimen was identified based on morphological and microscopic characteristics and compared with standard literature and herbarium records [18]. A voucher specimen of the authenticated plant material was prepared and deposited in the herbarium for future reference and documentation [21]. Proper collection and authentication of medicinal plants are essential steps for ensuring quality, purity, reproducibility and therapeutic efficacy of herbal formulations [20].

Analytical Pharmacognosy:

A. Macroscopic Characterization of Plant Material

Macroscopic evaluation was carried out to identify the crude drug based on external morphology and organoleptic properties [18,22].

a) Organoleptic Characters:

The plant material were evaluated using sensory observation methods [22].

Colour, Odour, Taste, Size, Shape.

b) Extra Features:

Special external features such as surface characteristics, arrangement of floral parts, and texture were observed and recorded [22].

c) Qualitative Macromorphology:

Morphological parameters such as length, width, diameter, shape, and surface appearance of plant materials were measured using a scale and visually examined [24].

B. Microscopic Characterization:

Material:

Instrument – Digital microscope and compound microscope.

Chemicals – Glycerine, Microchemical staining reagent like Phloroglucinol, HCL, Iodine etc.

a) Powder Microscopy Examination:

The powdered drug was treated with glycerine water and take in watch glass then added 1-2 drops reagent and spread it gently and covered with coverslip. The slide is observed under compound microscope at 10X and 40X to identify diagnostic characters such as fibres, trichomes, starch grains, and calcium oxalate crystals [23].

Physicochemical Investigation:

The physicochemical characters such as ash value, acid insoluble ash, water soluble ash, extractive value of powder was carried out as per standard methods.

a) Ash Value:

Ash value mainly used for the determination of purity and quality of drug.

1. Determination of total ash value:

Weight the crucible and heat it with burner on tripod stand. Weight about 2 gm of fine powder of air dried drug and pour it into crucible and strongly heat it with non-sooty flame to avoid carbon or burnt the carbon. Heat the crucible for minimum 2 hrs to get total ash of drug. Cool the crucible. Then, weight the ash and calculate the percentage total ash.

2. Determination of acid insoluble ash:

Take a crucible which contain total ash. Then add about 25 ml dil. HCL into it and boil it for 5 minutes. Filter the above solution of ash and HCL through Whatman filter paper and then wash it with water until the residue it get neutral. Collect the residue which is remain on filter paper called as acid insoluble ash and transfer it in crucible. Heat the crucible for 5 min to dry the ash residue. Cool the crucible and weight. Calculate the acid insoluble ash value with reference to air dry sample powder.

3. Determination of water soluble ash:

Take a crucible which contain total ash. Add about 25 ml water into it and boil it for 5 min. Filter the above solution of ash with Whatman filter paper and wash with warm water. Collect the residue which is remain on filter paper and transfer it in crucible. Heat the crucible for 5 min to dry the ash residue. Cool the crucible and weight. Calculate the water soluble ash value with reference to air dry sample powder.

b) Extractive Value:

Water soluble and alcohol soluble extractive values were determined to evaluate the amount of active constituents present in the plant material [26].

1. Water soluble extractive value:

Take 5 gm of air dried powder drug in a conical flask with stopper. Add 25 ml distilled water in conical flask which contain powder drug. Shake it for 6 hours and place it for 18 hours. Filter the solution and transfer the 25 ml of the filtrate in thin porcelain dish. Heat to the dryness and for complete drying use oven for constant weight. Cool the dish and weight. Then calculate the water soluble extractive with reference to air dried powder drug.

2. Alcohol soluble extractive value:

Take a 5 gm of air dried powder drug in a conical flask with stopper. Add 25 ml alcohol (Ethanol) in conical flask which contain powdered drug. Shake it for 6 hours and place it for 18 hours. Filter the solution and transfer the 25 ml of the filtrate in thin porcelain dish. Heat on water bath to the dryness and for complete drying use oven for constant weight. Cool the dish and weight. Then calculate the alcohol soluble extractive with reference to air dried powder drug.

Preparation of Extract:

Material –

Instrument – Soxhlet apparatus.

Apparatus - Round bottom flask, heating mantle, etc.

Chemicals – Ethanol and distilled water.

A) Preparation of Chamomile Extract:

Collect fresh chamomile flowers and wash them with distilled water to remove dust and impurities [18]. Shade dry the flowers for 7–10 days at room temperature [18]. Grind the dried flowers into coarse powder using a mechanical grinder [24]. Pass the powder through sieve no. 40 to obtain uniform particle size [24]. Prepare hydroalcoholic solvent by mixing ethanol and distilled water in 70:30 ratio [18]. Weigh about 25 g of chamomile flower powder [24]. Place the powdered material in a muslin cloth or thimble of Soxhlet apparatus [24]. Transfer 100 ml of hydro-alcoholic solvent into the round bottom flask attached to Soxhlet apparatus [18]. Assemble the Soxhlet apparatus with condenser and heating mantle properly [18]. Heat the solvent at controlled temperature to allow continuous extraction process [18]. Continue extraction for 6–8 hours [24]. Allow the apparatus to cool after completion of extraction [24]. Collect the extract from round bottom flask and filter it using filter paper [3]. Concentrate the filtrate using water bath to remove excess solvent [3]. Store the concentrated semisolid extract in airtight container under refrigerated condition for further studies [3].

B) Preparation of Palash flower Extract:

Material –

Apparatus – Heating-mantle, round bottom flask.

Chemicals - Ethanol and distilled water.

Collect fresh Palash flowers and wash them thoroughly with distilled water to remove dust and impurities [18]. Shade dry the flowers at room temperature for 7–10 days [18]. Grind the dried flowers into coarse powder using a mechanical grinder [24]. Pass the powder through sieve no. 40 to obtain uniform particle size [24]. Prepare hydroalcoholic solvent by mixing ethanol and distilled water in 70:30 ratio [18]. Weigh about 25 g of coarse powdered Palash flower material [24]. Transfer the powdered material into a clean beaker or round bottom flask [24]. Add 200 ml of hydro-alcoholic solvent to the powdered material [18]. Heat the mixture gently on heating mantle or water bath for about 30–45 minutes with continuous stirring [18]. Allow the mixture to cool at room temperature [24]. Filter the extract using muslin cloth or Whatman filter paper to remove plant debris [3]. Concentrate the filtrate on water bath to remove excess solvent and obtain semisolid extract [3]. Store the prepared extract in airtight container under refrigerated conditions for further use [3].

C) Preliminary Investigation of Extract:

The prepared extracts were evaluated for colour, odour, appearance, consistency, and nature of extract [3].

Phytochemical Investigation:

Phytochemical screening of *Matricaria Chamomilla* and *Butea Monosperma*:

Preliminary phytochemical screening of *Matricaria Chamomilla* extract and *Butea Monosperma* flower extract was carried out using standard qualitative chemical tests to identify the presence of various phytoconstituents such as alkaloids, flavonoids, tannins, glycosides, saponins, phenolic compounds, proteins, carbohydrates, and terpenoids [18].

Procedure:

Take small quantity of *Matricaria Chamomilla* extract and *Butea Monosperma* flower extract separately in clean test tubes [18]. Dissolve the extracts in suitable solvent such as distilled water or ethanol [27]. Perform different phytochemical tests for identification of chemical constituents [18].

Material –

Instruments / Apparatus – Burner, Glass test tube, Test tube holder, Test tube stand.

Chemicals – Conc.H₂SO₄, glacial acetic acid, HCL, dil. Iodine solution, lead acetate solution, distilled water, sodium hydroxide solution, chloroform, ferric chloride solution, molisch reagent, etc.

Phytochemical Tests:

For *Matricaria Chamomilla* and *Butea Monosprema*:

A. Test For Flavonoids:

1. Shinoda Test:

Take 2ml extract in test tube. Add small quantity of magnesium turnings. Add few drops of conc. Hydrochloric acid. Development of pink or reddish colour indicates presence of flavonoids [27].

2. Lead Acetate Test:

Take 2ml of extract of flower in a clean test tube. Add few drops of 10% of lead acetate solution to the extract. Shake the test tube gently and observe the reaction. Formation of yellow coloured ppt indicates presence of flavonoids [18].

3. Alkaline Reagent Test:

Take 2ml of extract in a clean test tube. Add few drops of sodium hydroxide solution to the extract. Observe the formation of intense yellow colour. Add dil. Hydrochloric acid to the reaction mixture. Disappearance of yellow colour after addition dil acid confirms presence of flavonoids [27].

B. Test for Terpenoids:

1. Salkowski Test:

Take 2 mL of flower extract in a clean test tube [18]. Add 2 mL of chloroform to the extract and mix properly [27]. Carefully add 1–2 mL of concentrated sulfuric acid along the side of the test tube to form a separate layer [18]. Observe the reaction at the interface of two layers [27]. Formation of reddish-brown colour at the interface indicates presence of terpenoids in chamomile extract [27].

C. Test For Phenolic Compounds:

1. Ferric Chloride Test:

Add few drops of ferric chloride solution to the extract. Formation of dark blue or green colour indicates presence of phenolic compounds [18].

D. Test For Tannins:

1. Ferric Chloride Test:

Add 5% ferric chloride solution to extract. Blue-black or greenish-black colour indicates presence of tannins [18].

E. Test For Glycosides:

1. Keller-Killiani Test:

Add glacial acetic acid and ferric chloride solution to the extract. Carefully add concentrated sulfuric acid. Formation of brown ring indicates presence of glycosides [24].

F. Test for carbohydrates:

1. Molish Test:

Add Molisch reagent to extract followed by concentrated sulfuric acid. Formation of violet ring indicates carbohydrates [24].

Antimicrobial Activity:

Antimicrobial activity of *Matricaria Chamomilla* and *Butea Monosperma* was to be done by agar plant diffusion method by using bacterial culture such as *Staphylococcus aureus* also known as *S. aureus* which is gram positive bacteria.

Material –

Instruments – Autoclave, Incubator, Weighing balance, Laminar air flow, Colorimeter, Zone reader, Sonicator, etc.

Apparatus – Conical flask, petri plates, glass stirrer, beaker, measuring cylinder, glass spreader, micro pipette, etc.

Chemicals – Yeast extract, Beef extract, peptone, NaCl, agar, distilled water, alcohol, etc.

Procedure for preparation of nutrient agar plates:

1. Preparation of Nutrient Agar Medium:

Clean and dry all glassware properly before use. Measure 100 mL of distilled water using a measuring cylinder and transfer it into a 250 mL conical flask. Accurately weigh 2.8 g of nutrient agar powder using a digital balance. Add the weighed nutrient agar powder slowly into the distilled water. Heat the mixture gently on a hot plate while stirring continuously with a glass rod until the medium dissolves completely and a clear solution is obtained. Check the pH of the medium using a calibrated pH meter and adjust the pH to 7.2–7.4 if required using 0.1 N NaOH or 0.1 N HCl.

2. Sterilization of Medium:

Plug the mouth of the flask with non-absorbent cotton and cover it with aluminium foil. Sterilize the prepared nutrient agar medium in an autoclave at:

Temperature: 121°C

Pressure: 15 psi

Time: 15–20 minutes [5]

After sterilization, remove the flask carefully and allow the medium to cool to approximately 45–50°C.

3. Preparation of Nutrient Agar Plates:

Switch on the laminar airflow chamber 15–20 minutes before use and disinfect the surface using 70% ethanol.[6] Arrange sterile Petri plates inside the laminar airflow chamber. Pour approximately 15–20 ml of molten nutrient agar medium into each sterile Petri plate aseptically. Allow the plates to remain undisturbed until the agar solidifies completely. After solidification, invert the plates to prevent condensation on the agar surface. Store the prepared agar plates at 4°C until further use.

Formula For nutrient Agar:

Sr.No	Ingredient	Quantity taken
1.	Beef extract	2.4gm
2.	Yeast extract	4.7 gm
3.	Peptone	9.4 gm
4.	Sodium chloride	10.0 gm
5.	Agar	23.5 gm
6.	Distilled Water	1000ml
7.	pH after sterilization	6.1

Table 1: Formulation table for preparation of nutrient agar

Procedure for Antimicrobial Activity by Agar Well Diffusion Method:

1. Preparation of Bacterial Inoculum:

Transfer bacterial culture of Staphylococcus aureus into sterile nutrient broth. Incubate at 37°C for 18–24 hours to obtain fresh bacterial growth.[8]

2. Incubation of Agar Plates:

Dip a sterile cotton swab into the bacterial suspension. Remove excess inoculum by pressing the swab against the wall of the test tube. Spread the bacterial culture uniformly over the entire surface of nutrient agar plate by rotating the plate at 60° angles to obtain even distribution.[9] Allow the inoculated plates to dry for 5 minutes.

3. Formation of Wells:

Using a sterile cork borer, make wells of approximately 6–8 mm diameter in the agar medium. Remove the agar plugs carefully using sterile forceps.

4. Addition of Face Serum Sample:

Fill each well with a measured quantity (50–100 µL) of face serum using a micropipette. One well may be filled with standard antibiotic solution as positive control and another with solvent as negative control.[10]

5. Incubation: Incubate the plates in inverted position at 37°C for 24 hours. After incubation, observe the plates for the formation of clear zones around the well.

Development and Optimization of Topical Formulation from Extract of *Matricaria Chamomilla* and *Butea Monosperma*.

A. Preparation of novel formulation form hydro alcoholic extract of Chamomile and Palash flow by 22 factorial design using Design Expert software.

B. Preparation and evaluation of face Serum.

Material –

Apparatus- Beaker, Water bath, Measuring cylinder, Weighing balance, Stirrer, Saptula.

Chemicals- Alcohol, Vitamin E, Tea tree oil, Lavender oil, Chamomile and Palashh extract, Almond oil, Oleic acid, Glycerine, Sodium Metabisulfide, Tween 80, PEG 400, Triethanolamine, Distilled Water, etc.

Selection of factors and levels for nano-emulsion:

The oil phase and surfactant mixture is selected as two factors which futher analysed the effect of factor on nanoemulsion by DoE.

Two levels are selected for formulation of nanoemulsion, these are as follows:

Factors	Low level	High level
Oil mixture (oilphase)	8ml	11ml
Smix (surfactant mixture)	15ml	20ml

Formulation Table:

Ingredients		FormulationCode(Quantity Taken)			
		F1	F2	F3	F4
Oil mixture (Oil phase)	Almond oil	5 ml	7 ml	5 ml	7 ml
	Oleic acid	3 ml	4ml	3 ml	4 ml
Smix (Surfactant mixture)	Tween80	10 ml	10 ml	13 ml	13 ml
	PEG400	5 ml	5 ml	7 ml	7 ml
Chamomile extract		2.5ml	2.5ml	2.5ml	2.5ml
Palash extract		2ml	2ml	2ml	2ml
Teatree oil		0.5ml	0.5ml	0.5ml	0.5ml
Lavender oil		0.5ml	0.5ml	0.5ml	0.5ml
Vitamin E		0.5ml	o.5ml	0.5ml	0.5ml
Glycerine		5ml	5ml	5ml	5ml
Sodium Metabisulfide		0.05gm	0.05 gm	0.05 gm	0.05 gm
Triethanolamine		0.3ml	0.3ml	0.3ml	0.3ml

Carbopol	0.5 gm	0.5 gm	0.5 gm	0.5 gm
Distilled water	65ml	63ml	60ml	57ml

Table 2: Formulation table for preparation of face serum (Batch F1, F2, F3 and F4).

Method of Preparation:

1. Preparation of Plant Extracts:

The dried flowers of Chamomile and dried flowers of Palashh are powdered separately. The powdered material is extracted using hydroalcoholic solvent by Soxhlet extraction method. The extracts are filtered and concentrated using a rotary evaporator. The concentrated extracts are stored in airtight containers for further use.

2. Preparation of Oil Phase:

Accurately weighed quantities of almond oil, lavender oil, tea tree oil, oleic acid, and vitamin E are mixed together in a clean beaker. The oil phase is heated mildly at about 40–45°C with continuous stirring until a uniform mixture is obtained.

3. Preparation of Aqueous Phase:

In another beaker, glycerin, Tween 80, PEG 400, sodium metabisulfite, and purified water are mixed properly. Triethanolamine is added dropwise to adjust the pH of the formulation near skin pH (5.5–6.5). The aqueous phase is also heated to the same temperature as the oil phase.

4. Formation of Nanoemulsion:

The herbal extracts are added into the aqueous phase with continuous stirring. The oil phase is then added slowly into the aqueous phase under magnetic stirring at high speed to form a coarse emulsion. Further reduction in globule size is achieved by high-speed homogenization at 10,000 rpm for 30 min to obtain a stable Nano-emulsion.

5. Preparation of Face Serum:

The prepared nano-emulsion is cooled to room temperature and checked for uniformity and then added in carbapol gel and again homogenized for 30 min. The final serum is transferred into clean airtight serum containers and stored at room temperature for further evaluation.

Evaluation of face serum:

Organoleptic Evaluation:

The prepared herbal nano-emulsion face serum is evaluated for its physical appearance such as color, odor, texture, homogeneity, consistency, and phase separation by visual inspection. The formulation should appear smooth, uniform, and free from grittiness.

1. pH Determination:

The pH of the serum is determined using a calibrated digital pH meter. About 1 g of serum is dispersed in distilled water and the electrode is immersed into the formulation. The pH should be maintained near skin pH (5.5–6.5) to avoid skin irritation.

2. Viscosity Determination:

Viscosity of the serum is measured using a Brookfield viscometer using suitable spindle at specific rpm. The formulation should possess optimum viscosity for easy application and spreadability on skin.

3. Washability Test:

The applied serum is washed with water to determine ease of removal from skin surface.

4. Spreadability Test:

Parallel-Plate Method A specific amount of cream (usually 0.5 g) is placed on a glass plate or petri dish. A series of known weights are carefully placed on the top glass plate at 1-minute intervals. The diameter of the spread circle is measured in centimeters. A standard formulation is generally expected to spread to a diameter between 5.0 cm and 7.0 cm.

Spreadability (S) is typically calculated using the following formula:

$$S = M \times L / T$$

Where,

(M): Mass applied to the top plate (in grams).

(L): Length that the product spreads (in mm or cm).

(T): Time taken for the sample to spread.

Particle Characterization:

1. Globule Size Determination:

The average globule size of nanoemulsion is measured using zeta sizer or particle size analyzer. Smaller globule size improves stability and penetration of active constituents into the skin.

2. Polydispersity Index (PDI):

Polydispersity Index (PDI) is an evaluation parameter used to determine the uniformity of particle size distribution in nanoemulsion formulations. It was measured using Dynamic Light Scattering (DLS) technique. Lower PDI values indicate better homogeneity and stability of the formulation. A PDI value below 0.3 is considered acceptable for stable nanoemulsions.

2² Factorial Design for Oil Mix and Smix:

2² factorial design was used for optimization of the nanoemulsion-based herbal face serum formulation. In this design, two independent variables, namely Oil mixture (Factor A) and Smix concentration (Factor B), were studied at two levels: low level and high level. The design helps in evaluating the effect of both variables on formulation parameters such as particle size, polydispersity index, viscosity, and stability. A total of four formulations were prepared according to the factorial design. The optimized formulation was selected based on desirable evaluation parameters.

Results:

Plant collection and authentication:

Reference No: SGIPSRT/ 2025-26/ 289. Authentication result and procured receipt of *Matricaria Chamomilla* and *Butea Monosperma* has been attached.

Fig1: Butea Monosperma (Palash)



Fig.2. : Matricaria Chamomilla (Chamomile)



Analytical Pharmacognosy:

Organoleptic macro-morphology of Chamomilla Matricaria And Butea Monosperma-

Sr.No	Character	Chamomile	Palash
1.	Colour	Cream Yellow	Bright orange, red
2.	Odour	Pleasant	Slightly
3.	Appearance	smooth	Soft
4.	Shape	Small, Circular	Irregular, fleshy petals

Table 3: Macroscopic characters of Chamomile and Palash flower.

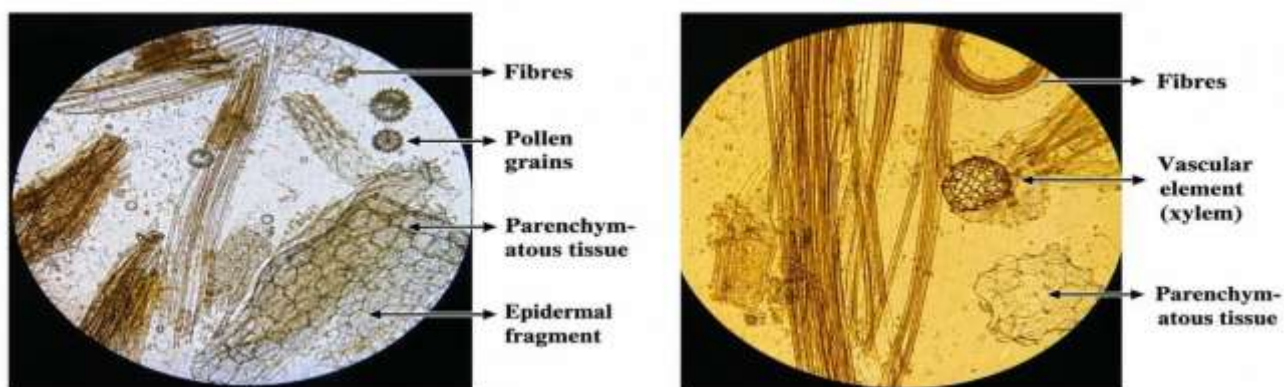
Microscopic Organoleptic characterization of powder:

Sr.no	Parameter	Chamomile flowers	Palash flowers
1	Color	Pale yellow to yellowish colored flowers	Flowers are bright orange red in color
2	Odor	Pleasant & aromatic	Odorless
3	Size	Tiny small flowers	Curved & beak shaped flowers

Table 4: Microscopic Characters Of chamomile and Palash flower.

Observation under compound microscope:

Fig 3: Microscopic Characterization of Chamomile and Palash Flower Powder



Physicochemical Study:

Preliminary physico-chemical parameters of powder drug.

Ash Value:

Parameters	Chamomile flower	Palash flower
Total ash	9.2 % w/w	7.5 % w/w
Acid in soluble ash	1.9 % w/w	1.7 % w/w
Water soluble ash	4.6 % w/w	3.8 % w/w

Table 5: Ash value of Chamomile and Palash Powder.

Extractive Value:

Parameter	Chamomile flower	Palash flower
Ethanol soluble extractive value	15 % w/w	14 % w/w
Water soluble extractive value	21 % w/w	20% w/w

Table 6: Extractive value of Chamomile and Palash flower

Preliminary investigation of chamomile and Palash extract:

Sr.No	Character	Chamomile extract	Palash extract
1.	Ethanollic extract		
	Nature	Liquid	Liquid
	Colour	Cream yellow	Bright orange-red
	Odour	Pleasant	Mild, Slightly
	Appearance	Clear liquid	Clear liquid
2.	Aqueous extract		
	Nature	Liquid	Liquid
	Colour	Dark brown	Brown
	Odour	Pleasant, aromatic	Mild, Slightly
	Appearance	Semi-solid	Semi-solid

Table 7: Preliminary investigation of chamomile and Palash extract.

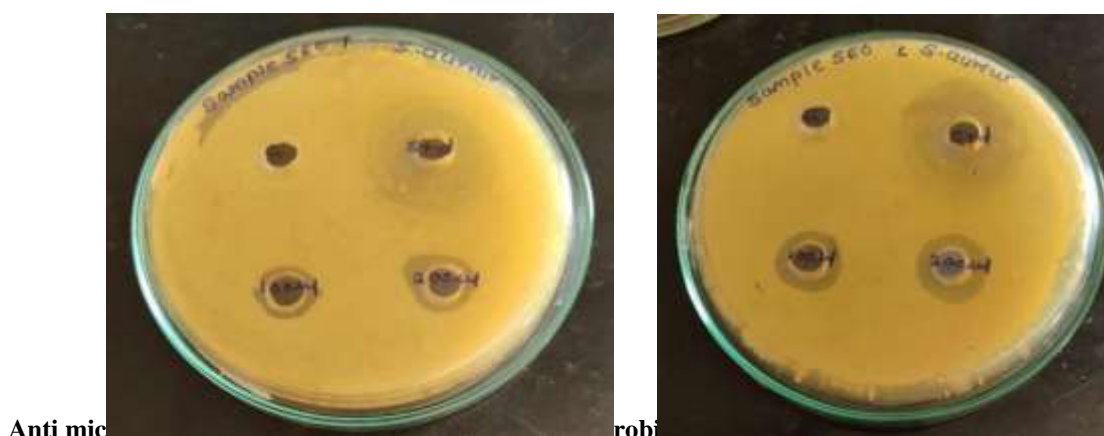
Phytochemical Investigation:

Phytochemical screening of Chamomile and Palash extract:

Sr.No	Phytochemical Constituent	Chamomile extract	Palash extract
1.	Alkaloids	-	-
2.	flavonoids	+	+
3.	Phenolic compound	+	+
4.	tannins	+	+
5.	saponins	+	+
6.	glycosides	+	+
7.	terpenoids	+	+

Table 8: Phytochemical screening of chamomile and Palash extract.

Antimicrobial Activity:



Anti mic

robi

Fig 4



Fig 5: Antimicrobial activity of Chamomile
Antimicrobial Activity of Formulation (P1,P2,C1,C2) :

Sr.No	Sample	Concentration	Zone of Inhibition (mm) S.aureus
1.	Control		-
2.	Standard Streptomycin	1 mg/ml	32
3.	Sample-P1	5 mg/ml	05
		10 mg/ml	08
4.	Sample-P2	5 mg/ml	04
		10 mg/ml	12
5.	Sample-C1	5 mg/ml	03
		10 mg/ml	07
6.	Sample-C2	5 mg/ml	05
		10 mg/ml	14

Table 9: Antimicrobial activity of aqueous and ethanolic extract

Sample P1 & P2 are Palash extract contain 1% & 2% concentration and C1 & C2 are Chamomile flower extract contain 1.5% & 2.5% concentration. As the concentration of extract increases the zone of inhibition was increases and show more anti-microbial activity. Hence P2 & C2 contain 2% and 2.5 % extract concentration having more antimicrobial activity as per results which show more zone of inhibition and these concentration was selected in formulation development.

Preparation and Evaluation of Herbal Nanoemulsion Based Face Serum:

Fig 6: Prepared Formulations of Nano-emulsion Based Face Serum



(F1, F2, F3, F4)

Organoleptic Evaluation of Face Serum:

Parameter	Formulation Code			
	F1	F2	F3	F4
Colour	Off white	Off white	Off white	Off white
Odour	Pleasant	Pleasant	Pleasant	Pleasant
Appearance	Smooth, Milky	Smooth, Milky	Smooth, Milky	Smooth, Milky
Homogeneity	Homogenous	Homogenous	Homogenous	Homogenous

Table 10: Organoleptic evaluation of face serum

pH of Face Serum:

Sr.no.	Formulation Code	pH
1.	F1	6.07
2.	F2	5.89
3.	F3	5.70
4.	F4	6.20

Table 11: pH of face serum

Viscosity of Face Serum:

Sr.no.	Formulation Code	Viscosity (cpc)
1.	F1	1556
2.	F2	2850
3.	F3	1202
4.	F4	2240

Table 12: Viscosity of Face Serum.

Spreadability of Face Serum:

Sr.no.	Formulation Code	Diameter Spread (cm)	Time Taken (min)	Spreadability (g.cm / sec)
1.	F1	5.1	1	12.25
2.	F2	5.6	1	16.00
3.	F3	6.5	1	18.00
4.	F4	5.3	1	13.25

Table 13: Spreadability of Face Serum.

Washability of Face Serum:

Sr.no	Formulation Code	Observation
1.	F1	Easily washable without residue
2.	F2	Easily washable without residue
3.	F3	Easily washable without residue
4.	F4	Easily washable without residue

Table 14: Washability of Face Serum

Particle characterization:

Globule Size of Face Serum:

Sr.no	Formulation Code	Globule Size(nm)
1.	F1	175.1
2.	F2	156.7
3.	F3	164.1

4.	F4	159.1
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Table 15: Globule Size of Face Serum.

Polydispersity Index (PDI):			
Sr.no	Formulation Code	Polydispersity Index	Observation
1.	F1	0.297	Good uniformity of globule size
2.	F2	0.303	Moderate uniformity of globule size
3.	F3	0.219	Excellent uniformity and narrow size distribution
4.	F4	0.307	Moderate uniformity of globule size

Table 16: Polydispersity Index of Face Serum.

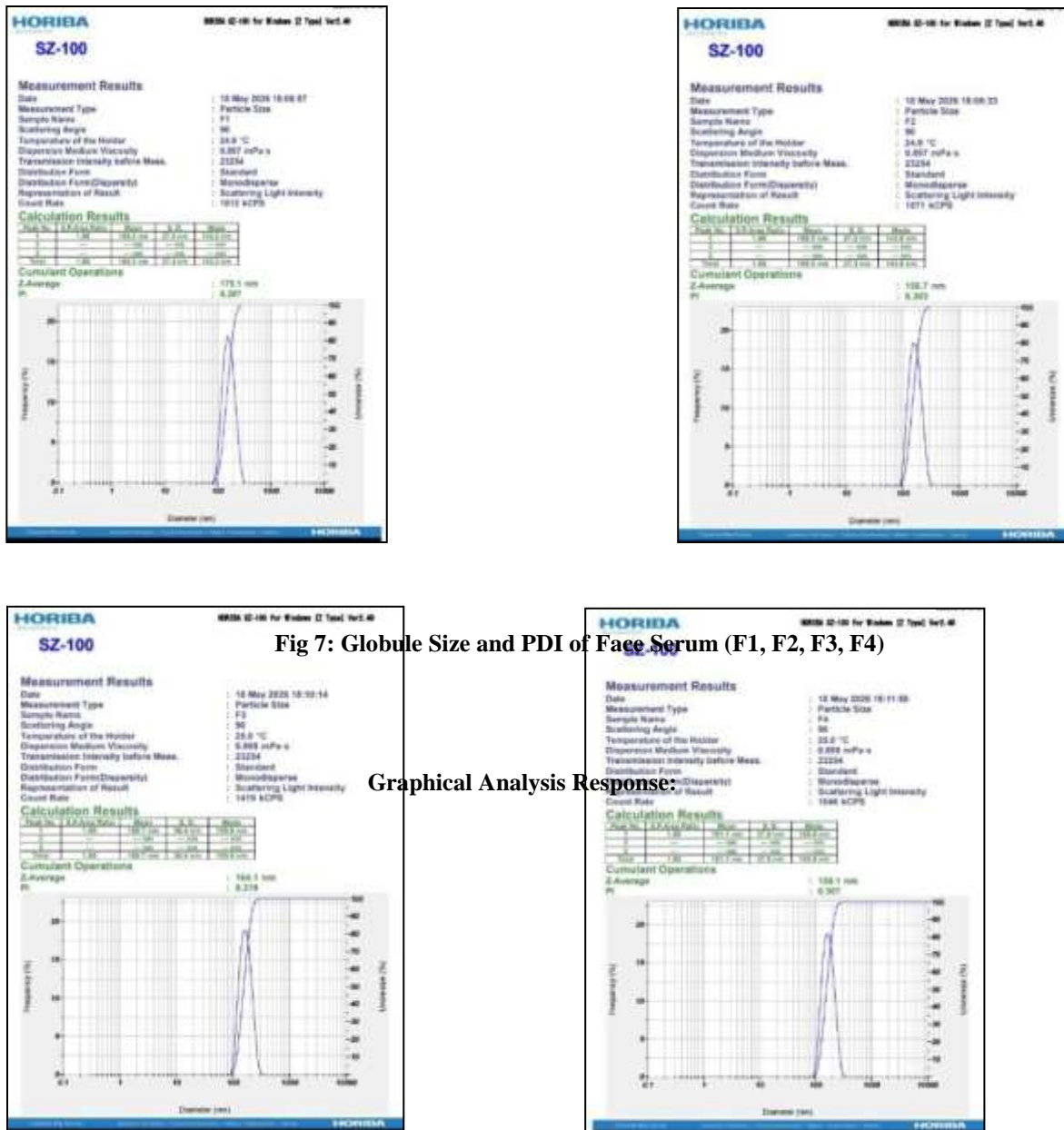


Fig 7: Globule Size and PDI of Face Serum (F1, F2, F3, F4)

Graphical Analysis Response:

Globule Size (nm) - Effect of Factor AB

Fig 8: Contour Plot of Effect of Factor AB on Globule Size (nm).

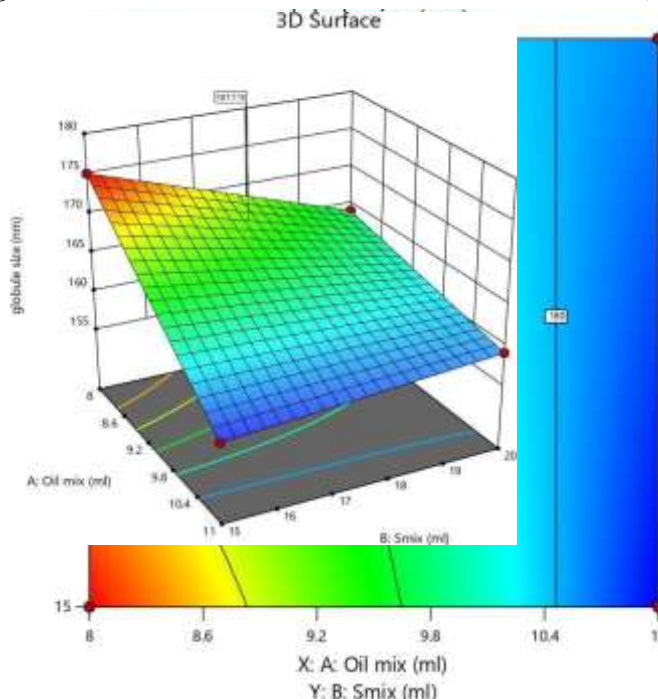


Fig 9: 3D Surface Graph of Effect of Factor AB on Globule Size (nm)

Final Formulated optimized batch(F3) with labeling:



Fig.10: Side and front view of containers

Conclusion:

The present study successfully formulated and optimized herbal nanoemulsion-based face serum using suitable herbal extracts and nano-emulsion technology. The antimicrobial activity of herbal extract was shown that antimicrobial activity increases with increase in concentration of extract. The prepared formulations showed good physical appearance, homogeneity, spreadability, pH, viscosity, and stability. Optimization using factorial design helped in selecting the optimized formulation with desirable globule size and polydispersity index. All formulations such as F1, F2, F3 & F4 show good and satisfactory results in acceptable limits.

Among all batches, the F3 formulation show good globule size, PDI, better nanoemulsion characteristics as compared to other 3 formulations. The nano-emulsion system enhanced the dispersion of herbal ingredients and improved the overall performance of the face serum. Therefore, the developed herbal nanoemulsion face serum can be considered a promising topical formulation for skincare applications such as acne reduction, moisturization, and skin protection.

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