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Formulation And Development Of Polyherbal Face Mist

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ABSTRACT

The increasing demand for multifunctional skincare products has driven the development of innovative formulations that address multiple skin concerns. This study focuses on the formulation and evaluation of a face mist incorporating both anti-acne and anti-wrinkle properties.

The mist was developed using a synergistic combination of herbal extracts, such as tea tree oil and lemongrass extract, known for their antimicrobial and antioxidant effects, along with Neem and liquorice extract to enhance skin hydration and reduce fine lines. The formulation was designed to be lightweight, non-comedogenic, and suitable for daily use. Physicochemical properties such as pH, and spray pattern were optimized for stability and ease of application. In vitro and in vivo assessments demonstrated promising results in reducing acne lesions and improving skin elasticity. The final product presents a convenient, effective skincare solution that supports clear and youthful skin, representing a novel contribution to the cosmeceutical market. The formulation process involved the extraction of bioactive compounds from neem leaves, liquorice and lemongrass using an optimized hydroalcoholic extraction method to ensure the retention of key phytochemicals like nimbin, nimbidin, azadirachtin, quercetin, liquiritin, glabridin, glycyrrhizin, citral, limonene and geraniol. These compounds are known to possess potent antimicrobial and soothing properties beneficial for facial

skin. The extract was then incorporated into a water-based mist formulation containing natural solvents, emulsifiers, preservatives (like Geogard ECT), and optional skin-conditioning agents such as glycerin for enhanced user comfort and skin hydration.

The prepared face mist was subjected to preliminary physicochemical evaluations, including pH stability, viscosity, microbial load testing, and organoleptic properties (color, odor, clarity). In vitro antimicrobial activity was assessed against common acne-causing bacteria such as *Staphylococcus aureus* and *Propionibacterium acnes*, revealing promising inhibitory effects. Patch testing on volunteers showed no signs of irritation or adverse reactions, supporting its suitability for topical use.

Objective:

To formulate and evaluate a herbal face mist using polyherbal extract for its potential antibacterial, anti-inflammatory, and skin-soothing properties, aiming to provide a natural, chemical-free skincare solution for managing acne, excess oil, and skin irritation.

1. Utilize natural phytochemicals known for their antibacterial, anti-inflammatory, antioxidant, and skin-brightening properties.
2. Minimize ethanol content ($\leq 8\%$) to ensure safety and compatibility with sensitive skin, while retaining preservative and solubilizing efficacy.
3. Incorporate green preservatives and solubilizers (e.g., Geogard ECT, Polysorbate 20/40) to enhance the stability and bioavailability of essential oils in an aqueous base.
4. Evaluate physicochemical parameters including pH, viscosity, density, sprayability, and microbial stability to ensure product safety, usability, and shelf-life.
5. Support the growing demand for clean, herbal-based skincare by developing a formulation that is non-irritating, non-comedogenic, eco-friendly, and cosmetically appealing.

AIM:-

The primary aim of this study is to develop a stable, effective, and user-friendly face mist formulation that exhibits both anti-acne and anti-wrinkle properties. This involves selecting and incorporating suitable active ingredients—such as botanical extracts, essential oils, and bioactive compounds—with proven antimicrobial, anti-inflammatory, antioxidant, and collagen-boosting activities. The formulation will be designed to ensure optimal skin compatibility, non-comedogenicity, and ease of application. Furthermore, the study aims to evaluate the physicochemical characteristics, microbiological stability, and dermatological efficacy of the face spray through appropriate in vitro and in vivo testing methods. The ultimate goal is to create a multifunctional skincare product that promotes clearer, smoother, and more youthful-looking skin.

To develop and evaluate a novel face mist formulation with dual functionality that effectively targets acne and signs of aging, by incorporating scientifically selected active ingredients with antimicrobial, anti-inflammatory, antioxidant, and anti-aging properties for enhanced skin health and appearance.

Literature review :-

Face Mist Formulation of Polyherbal extract

The growing consumer demand for herbal cosmetics has led to a surge in research on plant-based skincare formulations. Medicinal plants such as *Azadirachta indica* (Neem), *Glycyrrhiza glabra* (Liquorice), *Cymbopogon citratus* (Lemongrass), and essential oils like Tea Tree oil (*Melaleuca alternifolia*) have shown considerable potential in dermatological applications due to their phytochemical richness and therapeutic effects.

1. Neem (*Azadirachta indica*) Neem is widely known for its antibacterial, antifungal, and anti-inflammatory activities. The presence of active compounds such as nimbidin, azadirachtin, and quercetin contributes to its efficacy in treating acne and skin irritation

Biswas, K. et al. (2002). Biological activities and medicinal properties of neem (*Azadirachta indica*). *Current Science*, 82(11), 1336–1345.

Subapriya, R., & Nagini, S. (2005). Medicinal properties of neem leaves: A review. *Current Medicinal Chemistry – Anti-Cancer Agents*, 5(2), 149–156.

2. Liquorice (*Glycyrrhiza glabra*) Liquorice extract contains compounds such as glycyrrhizin, glabridin, and liquiritin, which exhibit skin-lightening, antioxidant, and anti-inflammatory properties.

Fu, B. et al. (2005). Anti-inflammatory and anti-oxidative effects of glycyrrhizin and glabridin in skin cells. *Planta Medica*, 71(9), 852–857.

Yoon, J. H. et al. (2010). Inhibitory effects of glabridin on melanogenesis. *Biological & Pharmaceutical Bulletin*, 33(3), 502–505.

3. Lemongrass (*Cymbopogon citratus*) Lemongrass is rich in citral (neral and geranial), which has antimicrobial, astringent, and antioxidant properties. It is also used for its pleasant citrus fragrance in cosmetics.

Shah, G. et al. (2011). Scientific basis for the therapeutic use of *Cymbopogon citratus* (DC) Stapf (Lemongrass). *Journal of Advanced Pharmaceutical Technology & Research*, 2(1), 3–8.

Oyediji, A. O. et al. (2009). Chemical composition and antimicrobial activity of the essential oil of *Cymbopogon citratus* from Nigeria. *African Journal of Biotechnology*, 8(1), 77–79.

4. Tea Tree Essential Oil (*Melaleuca alternifolia*) Tea tree oil is well known for its terpinen-4-ol content, which imparts potent antimicrobial and acne-fighting properties.

Carson, C. F., Hammer, K. A., & Riley, T. V. (2006). *Melaleuca alternifolia* (Tea Tree) oil: a review of antimicrobial and other medicinal properties. *Clinical Microbiology Reviews*, 19(1), 50–62.

Lee, H. S. et al. (2008). Terpinen-4-ol: A component of Tea Tree oil, suppresses inflammatory mediators in acne. *Archives of Dermatological Research*, 300(3), 125–132.

5. Herbal Cosmetics and Face Mists Several studies have emphasized the potential of herbal formulations in topical cosmetics due to their biocompatibility, minimal side effects, and therapeutic properties.

Dureja, H. et al. (2005). Cosmeceuticals: An emerging concept. *Indian Journal of Pharmacology*, 37(3), 155–159.

Mukherjee, P. K. et al. (2011). Evaluation of herbal products: Challenges and opportunities. *Pharma Times*, 43(5), 19–23.

PLAN OF WORK

Plan of Work for Herbal Face Mist Formulation

1. Literature Review

Study previous research on Neem (*Azadirachta indica*), Liquorice (*Glycyrrhiza glabra*), Lemongrass (*Cymbopogon citratus*), and Tea Tree Oil (*Melaleuca alternifolia*).

Review herbal cosmetics formulation strategies, solubilizer systems, and preservative mechanisms.

Explore regulatory limits on ethanol content and safe preservative usage in cosmetics.

2. Collection and Authentication of Raw Materials

Procure dried plant materials or standardised extracts (Neem, Liquorice, Lemongrass).

Obtain Tea Tree essential oil of cosmetic-grade purity.

Authenticate plant materials by a qualified botanist or pharmacognosy department.

3. Preparation of Extracts

If using raw materials:

Extract Neem and Liquorice using hydroethanolic solvent (30:65:5 Ethanol:Water:Glycerin).

Extract Lemongrass using ethanol:water (70:30), followed by evaporation of ethanol to reduce volatility and improve dermal safety.

Filter and store extracts in amber glass containers at 4°C.

4. Phytochemical Screening

Perform qualitative phytochemical tests on each extract:

Neem: Shinoda, Salkowski, FeCl_3 test

Liquorice: Foam test, Alkaline reagent test, Keller-Killiani, FeCl_3

Lemongrass: Sudan III test, Salkowski, Lead acetate, Shinoda test

Document presence of alkaloids, flavonoids, terpenoids, tannins, saponins, glycosides, essential oils, etc.

5. Formulation of Face Mist

Prepare three variants with different extract concentrations (Low, Medium, High).

Ingredients per 50 ml batch:

Neem, Liquorice, Lemongrass Extracts (with adjusted ethanol content)

Tea Tree Essential Oil (5 drops)

Solubilizer (e.g., Polysorbate 20 or 40)

Preservative (Geogard ECT, ECO, or alternative like Methyl Paraben)

Optional: Glycerin for added hydration

Maintain ethanol content $\leq 8\%$.

6. Evaluation of Final Formulations

7. Statistical Analysis

Evaluate formulation performance across variants.

Apply statistical tools like ANOVA for comparative evaluation.

8. Documentation and Reporting

Interpret results with respect to safety, efficacy, and consumer acceptability.

Prepare manuscript sections: Abstract, Introduction, Materials & Methods, Results, Discussion, Conclusion.

Cite all sources using appropriate referencing style (APA/Vancouver).

INTRODUCTION

In recent years, there has been a growing demand for herbal and natural-based cosmetics due to increased consumer awareness of synthetic ingredient side effects and a preference for clean, sustainable beauty. Among these, face mists have gained popularity as multifunctional skincare products, offering hydration, toning, antioxidant protection, and anti-inflammatory benefits through light, sprayable formulations ideal for daily use.

Skin care formulations are rapidly evolving, focusing on gentle, natural, and sustainable ingredients. Among these, face mists have emerged as essential hydrators and skin refreshers, especially for combination skin types. The development of a face mist with therapeutic herbal extracts like Neem (*Azadirachta indica*), Liquorice (*Glycyrrhiza glabra*), and Lemongrass (*Cymbopogon citratus*) optimized with low ethanol concentration offers a blend of functionality and safety. This project focuses on formulating a 50 ml face mist containing these botanical extracts, keeping ethanol content below 8% to maintain dermal safety while preserving antimicrobial and antioxidant efficacy.

Herbal actives derived from *Azadirachta indica* (Neem), *Glycyrrhiza glabra* (Liquorice), and *Cymbopogon citratus* (Lemongrass) possess a rich phytochemical profile, including flavonoids, saponins, glycosides, alkaloids, essential oils, and terpenoids, which confer antimicrobial, anti-inflammatory, and antioxidant effects. These properties are particularly beneficial in addressing concerns of combination skin, which often presents a mix of oiliness, dryness, and sensitivity.

Tea Tree essential oil (*Melaleuca alternifolia*) further enhances the formulation with its well-documented antimicrobial and acne-fighting properties. However, incorporating essential oils into aqueous systems requires careful use of solubilizers such as Polysorbate 20 or 40, while natural preservatives like Geogard ECT or Preservative ECO ensure microbial stability without harsh additives.

The current study focuses on the formulation, evaluation, and optimization of a low-ethanol, gentle face mist that combines these herbal extracts for enhanced skin benefits. The ethanol content is restricted to $\leq 8\%$ to ensure dermal safety, especially for daily use. Three concentration variants were prepared to assess optimal extract balance and skin compatibility.

This research aims to bridge traditional herbal knowledge with modern cosmetic formulation techniques, producing a stable, effective, and aesthetically pleasant face mist suitable for commercial and dermatological application.

To use a face spray (also known as a facial mist or setting spray), follow these steps depending on the purpose of the spray:

1. For Hydration or Refreshing Your Skin

Steps:

Cleanse your face (optional if you're just refreshing).

Hold the spray 6–8 inches away from your face. Close your eyes and mouth, then spray evenly in an "X" or circular motion. Let it air dry or gently pat with clean hands. Use any time during the day to refresh or hydrate.

2. Before Makeup (Priming Spray)

Steps: Wash and moisturize your face.

Spray evenly on your face as above.

Let it dry completely before applying makeup.

3. After Makeup (Setting Spray)

Steps:

Finish your makeup completely.

Hold the bottle 6–8 inches away.

Spray in an "X" and then a "T" shape to cover your face evenly.

Let it air dry—do not rub.

Tips:

Always shake the bottle first if recommended on the label.

Don't overspray—2–4 spritzes are usually enough.

Store hydrating sprays in the fridge for a cooling effect.

Anatomy of Face And its Function :

The anatomy of the skin consists of three main layers, each with specific functions:

1. Epidermis (Outer Layer)

Structure: Made mostly of keratinocytes (cells that produce keratin).

Layers (from deepest to surface):

- Stratum basal (basal layer): site of cell division.
- Stratum spinosum

- Stratum granulosum
- Stratum lucidum (only in thick skin like palms and soles)
- Stratum corneum (outermost, dead cells)

Functions:

Barrier against pathogens.

Prevents water loss.

Produces melanin (via melanocytes).

2. Dermis (Middle Layer)

Structure: Dense connective tissue with collagen and elastin.

Two regions:

Papillary layer (superficial): loose connective tissue, capillaries, sensory neurons.

Reticular layer (deeper): thicker, contains sweat glands, sebaceous glands, and hair follicles.

Functions:

Supports epidermis.

Provides strength and elasticity.

Contains nerves and blood vessels.

3. Hypodermis (Subcutaneous Layer)

Structure: Mostly fat (adipose tissue) and connective tissue.

Functions:

Insulates body.

Stores energy.

Cushions internal organs.

Other Important Components

Hair follicles

Sebaceous (oil) glands

Sweat glands (eccrine and apocrine)

Nerve endings (for touch, pain, temperature)

Blood vessels (in dermis and hypodermis)

IDEAL PROPERTIES OF HERBAL FACE SPRAY

1. Skin Compatibility

– Non-irritating, hypoallergenic, and suitable for daily use, especially on combination or sensitive skin.

2. Low Ethanol Content ($\leq 8\%$)

– Ensures minimal drying effect while improving preservation and enhancing extract penetration.

3. Antimicrobial Activity

– Thanks to Neem, Tea Tree Oil, and Lemongrass, the mist helps prevent acne-causing bacteria and supports skin hygiene.

4. Anti-inflammatory Action

– Ingredients like Glycyrrhizin, Quercetin, and Nimbidin soothe inflamed or irritated skin.

5. Antioxidant Protection

– Rich in flavonoids and citral, it protects skin against oxidative stress and pollution.

6. Skin Brightening Effect

– Liquorice constituents (Glabridin, Liquiritin) help reduce pigmentation and enhance skin tone.

7. Hydration and Moisturization

– Vegetable glycerin and aqueous extracts provide lightweight hydration without heaviness.

8. Balanced pH (~5.0–6.0)

– Maintains the skin's natural acid mantle, reducing sensitivity and promoting barrier health.

9. Fine Spray Dispersion

– Even and pleasant misting from the spray bottle for user-friendly application.

10. Aromatic Freshness

– Natural citrus-herbal scent from lemongrass and tea tree oil enhances user experience.

11. Clear or Slightly Tinted Appearance

– Aesthetic clarity without sedimentation, turbidity, or phase separation.

12. Stable Shelf Life (≥ 3 –6 months)

– Preservative system ensures microbial stability and maintains extract activity.

13. Non-Comedogenic

– Lightweight, non-oily formula that does not clog pores, suitable for acne-prone skin.

14. Eco-friendly Ingredients

– Biodegradable, plant-based, and free from synthetic dyes, parabens, or sulfates.

15. Multifunctional Benefits

– Acts as a toner, refresher, antioxidant shield, and calming mist – all-in-one product.

PLANT PROFILE

1) Neem:

Synonyms: Neem, Nimtree, Indian Lilac

Biological name: *Azadirachta indica* A. Juss.

Family: Meliaceae

Organoleptic characteristics:

Color: Neem leaves are typically bright to dark green in color.

Odor: Neem leaves and oil have a strong, bitter, and slightly pungent aroma.

Taste: They possess a very bitter taste, often described as intensely astringent and lingering.

Texture: The leaves are elongated and serrated with a leathery texture; the seeds are small, smooth, and oval-shaped.

Uses of Neem (*Azadirachta indica*):

1. **Antibacterial and Antifungal Properties:** Neem is widely known for its potent antimicrobial effects. It helps in treating skin infections, acne, and other microbial conditions by killing harmful bacteria and fungi.
2. **Skin Soothing and Healing:** Neem extracts are used to soothe irritated skin, reduce inflammation, and promote wound healing, making it highly effective in herbal face sprays and topical applications.
3. **Oil Control:** Neem helps regulate excess sebum production, making it beneficial for oily and acne-prone skin.
4. **Antioxidant Protection:** Neem is rich in antioxidants, which protect the skin from environmental damage and premature aging.

2) LIQUORICE :

Synonyms: Licorice, Sweetwood, Yashtimadhu, Mulethi

Biological name: *Glycyrrhiza glabra* L.

Family: Fabaceae

Organoleptic characteristics:

Color: Dried roots are yellowish-brown on the outside and pale yellow inside.

Odor: Characteristic, sweet and pleasant.

Taste: Sweet, due to the presence of glycyrrhizin.

Texture: Roots are tough, fibrous, and cylindrical with longitudinal striations.

Uses of Liquorice:

Anti-inflammatory: Licochalcone A, another component, soothes irritated or red skin — useful for acne-prone or sensitive skin.

Antioxidant Protection

Helps neutralize free radicals and protect against environmental stressors.

Anti-bacterial

Supports clearer skin by reducing acne-causing bacteria.

Skin Brightening

Contains glabridin, which helps reduce hyperpigmentation and dark spots by inhibiting melanin production.

3) TEA TREE OIL :

Synonyms: Melaleuca oil, Ti Tree Oil, Melaleuca alternifolia oil.

Biological name: Melaleuca alternifolia

Family: Myrtaceae

Organoleptic characteristics:

Color: Tea tree oil is typically a pale yellow to nearly colorless clear liquid.

Odor: It has a strong, fresh, medicinal scent with camphoraceous and slightly spicy undertones.

Taste: Not typically consumed orally due to its potency and toxicity in high doses; however, it has a strong bitter taste.

Texture: As an essential oil, it has a thin, oily texture that spreads easily over the skin.

Uses of Tea Tree Oil:

Antibacterial & Antimicrobial Action: Tea tree oil helps reduce acne-causing bacteria on the skin, promoting clearer skin.

Anti-inflammatory Benefits: Reduces redness, swelling, and inflammation from acne or irritation.

Skin Refreshment: Provides a cooling and refreshing feel to the skin, especially in combination with other ingredients like rose water or aloe vera.

Oil Control: Helps balance skin's natural oils and reduce excess sebum production.

4. Lemongrass (Cymbopogon citratus)

Synonyms:-West Indian Lemongrass, Citronella grass (note: may also refer to Cymbopogon nardus) ,Sweet Rush ,Fever grass ,Bhustrina (Ayurveda)

Biological Source

Botanical Name: Cymbopogon citratus (DC.) Stapf

Family: Poaceae (Gramineae)

Part Used: Fresh or dried leaves, sometimes stems

Habitat: Native to India and Sri Lanka; widely cultivated in tropical and subtropical regions
Organoleptic Characteristics:

Appearance:- Long, Narrow, Blade-like green leaves

Odor:-Strong lemon-like citrusy fragrance

Taste:-Citrusy, slightly sharp and bitter

Texture:- Fibrous and grass-like

Color:- Pale green to yellowish (dried form)

Uses :-

1 .Anti-Cancer Effects

Compounds like citral in lemongrass have shown the ability to kill certain types of cancer cells by triggering natural cell death (apoptosis).

2. Anti-Biofilm Activity

Lemongrass oil helps stop bacteria from forming biofilms (a slimy layer that protects them), making it useful in treating skin infections and acne.

3. Natural Preservative

Due to its strong antimicrobial and antifungal action, lemongrass oil is being used as a natural preservative in food and cosmetic products, replacing harmful synthetic preservatives.

4. Aromatherapy and Stress Relief

The fresh, citrus scent of lemongrass is found to reduce stress and anxiety when used in diffusers, sprays, or oils. It may also help improve memory and focus.

5. Insect Repellent

Lemongrass oil is effective in repelling mosquitoes and insects. It is commonly used in eco-friendly sprays and creams as a natural alternative to chemical repellents.

6. Skin Barrier Repair

Lemongrass extract helps in improving the skin's moisture barrier and reduces dryness, thanks to its antioxidants and essential oils like geraniol.

FORMULA

Sr.No.	Ingredient	F1	F2	F3
1.	Neem Extract	6ml	6.5ml	7ml
2.	Liquorice Extract	6ml	6.5ml	7ml
3.	Lemongrass Extract	6ml	6ml	6ml
4.	Distilled Water	q.s.	q. s.	q.s.
5.	Tea Tree Oil	5 drops	5 drops	5drops
6.	Solublizer (Polysorbate)	2ml	2.5ml	2.5ml
7.	Preservative	0.5ml	0.5ml	0.5ml
8.	Glycerin	2.5ml	2.5ml	2.5ml

MATERIALS AND METHODS**Materials**

The following raw materials were used for the preparation and evaluation of the polyherbal face mist formulation:

Plant Extracts:

Neem (*Azadirachta indica*) extract – prepared using a 30:65:5 solvent ratio (Ethanol:Water:Glycerin)

Liquorice (*Glycyrrhiza glabra*) extract – prepared using a 30:65:5 solvent ratio

Lemongrass (*Cymbopogon citratus*) extract – extracted using 70:30 Ethanol:Water, then diluted 1:1 with water post-ethanol evaporation

Essential Oil:

Tea Tree Essential Oil – antimicrobial and acne-control agent

Other Ingredients:

Distilled Water – as diluent and hydration base

Solubilizer: Polysorbate 20 (or Polysorbate 40 LR)

Humectant: Glycerin (vegetable grade)

Preservative: Geogard ECT / Preservative ECO (a broad-spectrum, eco-certified preservative)

pH Buffer (optional): Citric acid or Sodium citrate (if adjustment is needed)

Analytical reagents: Mayer's reagent, FeCl_3 , Salkowski reagent, Sudan III, etc., for phytochemical analysis

Equipment

Electronic balance

pH meter

Brookfield viscometer

Autoclave

Laminar air flow cabinet

Petri plates and culture media (Nutrient agar, Soy broth, Sabouraud Dextrose Agar)

Magnetic stirrer

UV chamber

Graduated cylinders and volumetric flasks

Sterilized amber spray bottles (50 mL)

METHOD OF PREPARATION

1. Water Phase:

In a sterilized beaker, combine Neem Extract, Liquorice Extract, Lemongrass Extract, and Distilled Water.

Stir gently to ensure a uniform mixture.

2. Oil Phase

In a separate container, mix Tea Tree Essential Oil with the Solubilizer until a homogeneous solution is achieved.

3. Combine Phases:

Slowly add the oil phase to the water phase while continuously stirring to ensure proper emulsification.

4. Additives:

Incorporate the Preservative and Vegetable Glycerin (if using) into the mixture.

Stir thoroughly to ensure all components are well integrated.

5. Bottling:

Transfer the final mixture into a sterilized 50 ml amber spray bottle.

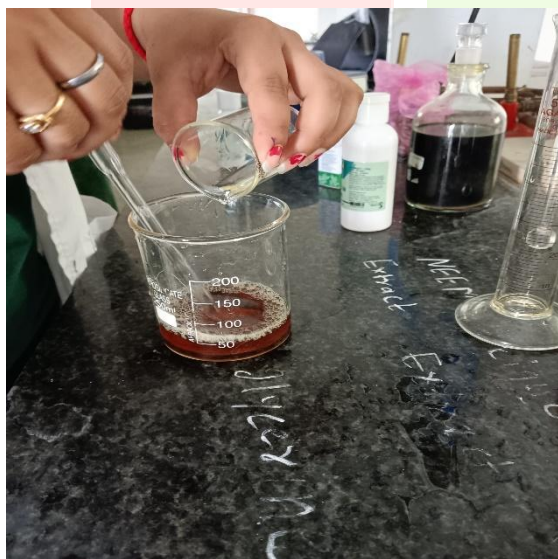
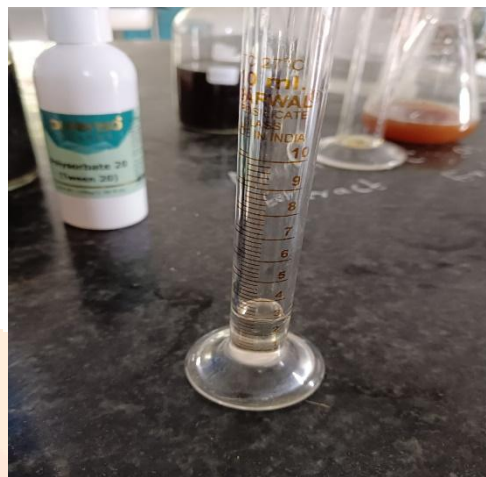
Label accordingly and store in a cool, dark place.

Usage Guidelines:

Application: Spritz onto the face after cleansing or as needed for a refreshing effect.

Storage: Keep in a cool, dark place to maintain efficacy.

Shelf Life: Use within 3 months. If a natural preservative is used, consider refrigeration to extend shelf life..





METHOD OF EXTRACTION:-

All the three extraction was carried out with the help of “Cold Maceration”

1].Neem Extraction

Grind the dried neem leaves into a coarse powder

1. Mix Solvent: Combine 240ml ethanol, 520ml water, and 40ml glycerin in a clean glass container.
2. Add Neem: Place 80g of Neem into the solvent mixture.
3. Maceration
Cold Maceration: Store for 7-14 days, shaking daily
4. Filtration: Strain through cheesecloth or a fine filter
5. Storage: Store in an amber glass bottle to protect from light.



FIG :- During neem extraction

2].Liquorice extraction

1. Mix Solvent: Combine 390ml ethanol, 845ml water, and 65ml glycerin in a clean glass container.
2. Add Liquorice: Place 130g of dried liquorice powder into the solvent mixture.
3. Maceration
Cold Maceration: Store for 7-14 days, shaking daily
4. Filtration: Strain through cheesecloth or a fine filter
5. Storage: Store in an amber glass bottle to protect from light



FIG:- During the extraction process

3] Lemongrass Extraction :-

1. Mix Solvent: Combine 160 ml ethanol and 240ml water in a clean glass container.
2. Add Lemongrass : Place 80g of Lemongrass into the solvent mixture.
3. Maceration
Cold Maceration: Store for 7-14 days, shaking daily
4. Filtration: Strain through cheesecloth or a fine filter
5. Storage: Store in an amber glass bottle to protect from light

Evaluation test for extract

1.TEST FOR NEEM EXTRACT:-

A] SALKOWSKI TEST:-

Mix 2ml of extract with 2ml of chloroform, then carefully add 2ml conc.H₂SO₄

Result:-Reddish brown interface forms if terpenoids present

It detects presence of NIMBIDIN, AZADIRACHTIN, AND NIMBIN

B] SHINODA TEST:-

Add a few magnesium turnings + 2-3 drops of concentrated HCL to 2ml extract.

Result:- Pink to red color appears

It detects the presence of Flavonoids (Quercetin)

C] FERRIC CHLORIDE TEST (FeCl_3):-

Add 2-3 drops of 5% FeCl_3 solution to 2 ml extract

Result:- Green, blue or black coloration indicates phenols or flavonoids

It detects the presence of Quercetin

USE OF THE EXTRACT CONSTITUENTS:-

CONSTITUENTS	USES
Nimbidin	Anti-inflammatory, Anti-microbial
Azadirachtin	Potent Anti-bacterial
Quercetin	Anti-Oxidant, Anti-aging
Nimbin	Anti-septic, Skin-soothing



FIG :- Test for neem extract

2. TEST FOR LIQUORICE EXTRACT

A] ALKALINE REAGENT TEST:-

Add 2ml of extract in test tube + Few drops of NaOH (Sodium Hydrochloride)

Result : Yellow color appears

It detects the presence of Liquiritin, Glabridin (flavonoids)

B] FOAM TEST

Shake 2ml extract with 10ml distilled water vigorously for 30 seconds, let it stand

Result – Persistent froth for or less than 10 minutes

It detects the presence of Glycyrrhizin.

C] KELLER-KILLIANI TEST

Add glacial acetic acid + FeCl_3 + H_2SO_4 in 2ml of extract

Result :- Reddish-brown ring at junction

USES OF THE EXTRACT CONSTITUENT

Constituent	Uses
Glycyrrhizin	Anti-inflammatory, Skin-soothing
Glabridin	Skin brightening, inhibits melanin synthesis
Liquiritin	Fades dark spots and pigmentation
Flavonoids	Anti-oxidant



FIG:- Test of Liquorice extract

3. LEMON GRASS EXTRACT TEST

A] SUDAN III TEST (for essential oils)

Mix 2ml extract + few drops of Sudan III solution and shake gently

Result : - Reddish-orange color appears if essential oils are present

It detect the presence of Geraniol and Citral

B] SALKOWSKI TEST

Add 2ml chloroform + 2ml concentrated H_2SO_4 to 2ml extract carefully and let it stand undisturbed

Result : - Reddish-brown ring between two layers confirms presence of terpenoids like limonene

It detect the presence of Limonene

C] LEAD ACETATE TEST

Add a few drops of 10% lead acetate solution to 2ml extract

Result :- Yellow precipitate indicates presence of flavonoids

USES OF THE EXTRACT CONSTITUENTS

Constituent	Uses
Citral	Anti-microbial, astringent ,fragrant
Limonene	Anti-oxidant, mildly exfoliating
Geraniol	Toning , Anti-microbial
Flavonoids	Anti-oxidant ,skin protective

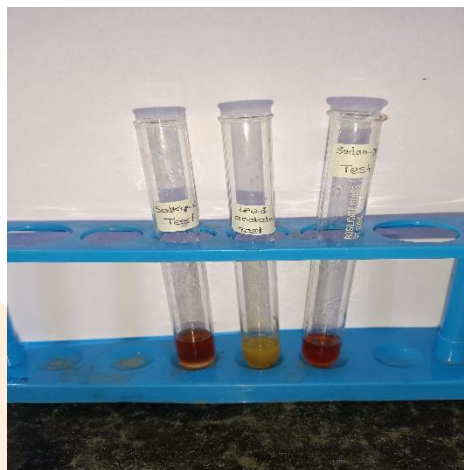


FIG:- Test of lemongrass extract

EVALUATION TEST FOR FORMULATION

A] ORGANOLEPTIC TEST :

Objective : The objective of the organoleptic test is to evaluate the sensory attributes of the herbal solution ,including its appearance ,color, odor, and over all acceptability ,ensuring it meets consumer expectation and provides a pleasant user experience.

1. Appearance :

The herbal formulation presents a clear to slightly hazy liquid.

Homogeneous and free from suspended particles.

No phase separation or sedimentation upon standing

2. Color:

Light yellow to pale green (due to natural extracts and essential oils)

May vary slightly depending on extract concentration and storage duration

3. Odor :

Fresh herbal citrus aroma

Distinct but pleasant scent due to lemongrass and tea tree oil

4. Texture

Light, non-sticky, quick-absorbing mist

Leaves a mildly hydrated and refreshed skin feel

No greasy residue

5. Sprayability:

Fine mist dispersion from the spray pump

Uniform coverage without clogging or splashing

6. After-feel:

Cooling, refreshing sensation

No stinging, irritation, or dryness on normal skin

Slight tightening/toning effect (from lemongrass and tea tree).



B] pH Determination

Purpose: To ensure the pH is suitable for facial skin (ideal range: 4.5–6.0).

Procedure:

Calibrate the pH meter using standard buffers (pH 4.0 and 7.0).

Measure 10 ml of the formulation.

Insert the electrode into the sample and record pH.

Frequency: Immediately after preparation and at intervals (7, 14, 28 days) to check stability.



FIG :- CALIBRATED pH METER



FIG:- after taking pH of face mist

RESULT:- The pH of face mist was found to be 5.04

C]. Viscosity Test

Purpose: To determine the flow and application behavior.

Procedure:

Use a Brookfield viscometer, Ostwald viscometer (Spindle No. 1, 10–20 rpm).

Measure viscosity at room temperature ($25 \pm 2^\circ\text{C}$).

Expected Result: Low-viscosity liquid (10–100 cP) suitable for misting.

Viscosity Calculation of Face Mist Formulation using Ostwald Viscometer

Formula used:

$$\eta_1 = \eta_2 \times (d_1 / d_2) \times (t_1 / t_2)$$

Where:

η_1 = viscosity of the sample (formulation)

η_2 = viscosity of water (standard)

d_1 = density of the formulation

d_2 = density of water

t_1 = time taken by formulation (in seconds)

t_2 = time taken by water (in seconds)

Given:

$$t_1 = 4.48 \text{ minutes} = 268.8 \text{ seconds}$$

$$t_2 = 1.00 \text{ minute} = 60 \text{ seconds (assumed)}$$

$$d_1 = (126 \text{ g} - 95 \text{ g}) / 10 \text{ mL} = 3.1 \text{ g/mL}$$

$$d_2 = 1.0 \text{ g/mL}$$

$$\eta_2 = 0.89 \text{ cP (viscosity of water at } 25^\circ\text{C)}$$

Substitute the values:

$$\eta_1 = 0.89 \times (3.1 / 1.0) \times (268.8 / 60)$$

$$\eta_1 = 0.89 \times 3.1 \times 4.48$$

$$\eta_1 = 12.36 \text{ cP}$$

Result:

The viscosity of the face mist formulation is 12.36 centipoise (cP).

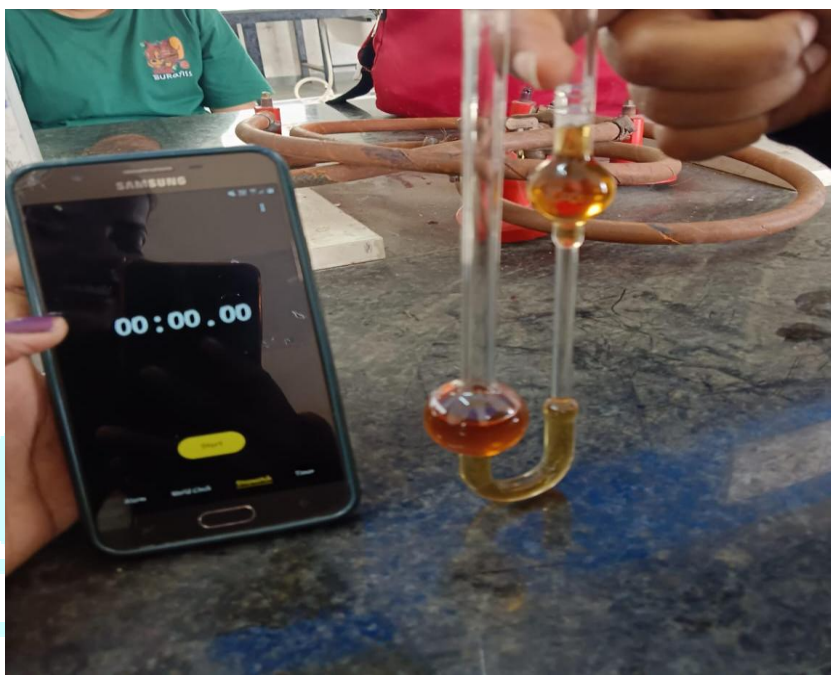


FIG :- BEFORE STARTING

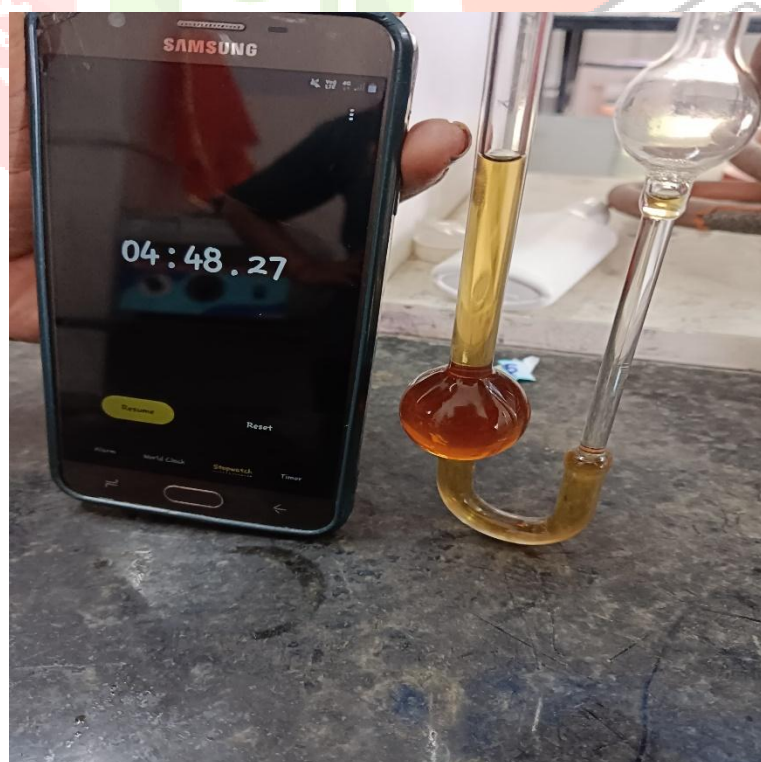


FIG :- AFTER IT TRAVELS TO END POINT

D] Sprayability test :- uniform spray without clogging

Purpose: To assess ease of application.

Procedure:

Fill the formulation in a standard spray bottle.

Spray 3–4 times from a distance of 15 cm onto a blotting paper.

Observe uniformity, spread, and any clogging.

RESULT:- Uniformly sprayed, spread without clog

E].Stability Testing (Accelerated)

Purpose: To evaluate stability under different storage conditions.

Conditions:

Room temperature ($25 \pm 2^\circ\text{C}$)

Accelerated ($40 \pm 2^\circ\text{C}$ and 75% RH)

Refrigerated ($4 \pm 2^\circ\text{C}$)

Duration: 30, 60, and 90 days.

Parameters: Color, odor, pH, phase separation, microbial load.

RESULT:- The polyherbal face mist was found to be stable for 3 to 4 month

F] Irritancy/Skin Compatibility Test (Patch Test)

Purpose: To ensure skin safety.

Procedure:

Apply 0.5 ml on a small area of inner forearm.

Cover with a patch for 24 hours.

Observe any signs of redness, itching, or irritation.

Volunteers: 10–15 healthy individuals (with consent).

Outcome: No visible signs of adverse reactions.



FIG :- APPLICATION OF FACE MIST



FIG :-AFTER 24 HOURS OF APPLICATION

RESULT :- No redness ,Rash and Side effect was observed

G] Microbial Load Test Procedure

Materials Needed:

Nutrient agar (for bacteria)

Sabouraud dextrose agar (for yeast & mold)

Sterile saline or phosphate buffer

Petri dishes

Incubator (30–35°C for bacteria; 20–25°C for fungi)

Sterile pipettes, dilution tubes

Face mist sample

Step-by-Step Procedure:

1. Sample Preparation:

Take 1 g or 1 mL of the face mist.

Dilute in 9 mL of sterile saline/buffer to make a 1:10 dilution.

Perform serial dilutions up to 1:1000 if needed.

2. Inoculation:

Plate 1 mL of each dilution onto:

Nutrient Agar (for Total Aerobic Bacterial Count)

Sabouraud Agar (for Yeast & Mold Count)

Pour melted, cooled agar (~45°C) into the plates and swirl gently.

4. Incubation

TAMC (Bacteria): Incubate at 30–35°C for 48–72 hours

TYMC (Yeast & Mold): Incubate at 20–25°C for 5–7 days

Incubate Nutrient Agar plates at 30–35°C for 48–72 hours.

Incubate Sabouraud Agar plates at 20–25°C for 5–7 days.

5. Colony Counting

After incubation:

Count the colony-forming units (CFUs) on plates that have 30–300 colonies.

Calculate the CFU/mL or CFU/g using this formula:

CFU/mL or CFU/g = Number of colonies × Dilution factor

Example: If you plated 1 mL from a 1:100 dilution and counted 45 colonies:

> CFU/mL = $45 \times 100 = 4,500$ CFU/mL

6. Acceptance Criteria (for cosmetics)

According to ISO 17516:

TAMC: $\leq 1,000$ CFU/g or mL

TYMC: ≤ 100 CFU/g or mL



FIG:- Before incubation (Nutrient agar medium for bacteria)

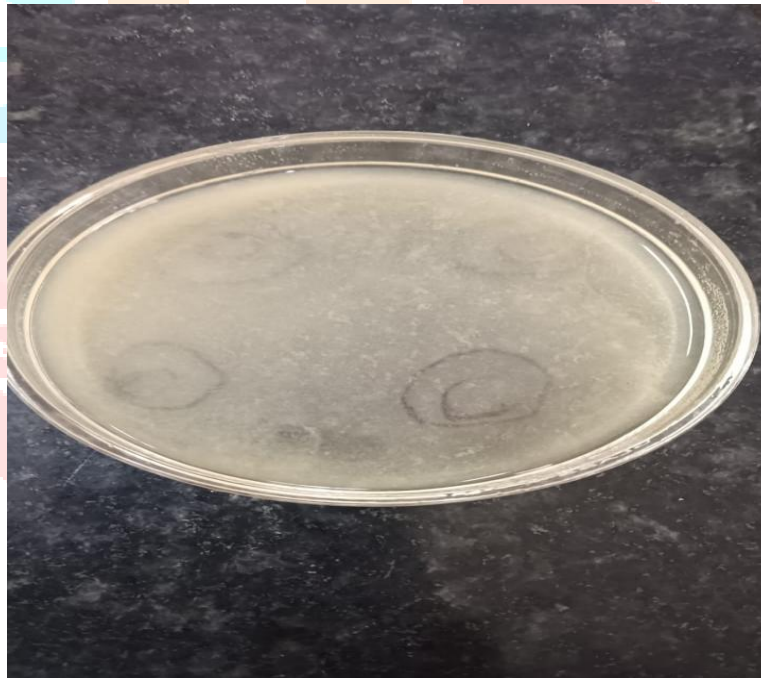


FIG :- After incubation (Nutrient agar medium for bacteria)



FIG :- Before incubation (Sabouraud Dextrose Agar for Fungi and Mold)

Microbial Load Test Results:

The prepared face mist formulation was subjected to microbial load analysis using Nutrient Agar (NA) for bacterial growth and Sabouraud Dextrose Agar (SDA) for fungal growth. Plates were incubated at $37 \pm 1^\circ\text{C}$ for 24–48 hours (for bacteria) and at $25 \pm 2^\circ\text{C}$ for 5–7 days (for fungi).

Observation:

No visible bacterial colonies were observed on the Nutrient Agar plates.

-Indicates satisfactory preservation and hygienic processing

No fungal or mold growth was seen on SDA plates during the incubation period.

Conclusion: The formulation meets acceptable microbiological standards for cosmetic products. This indicates the effectiveness of the preservative system and hygienic manufacturing conditions.

H] Pathogen-Specific Tests in Cosmetics

Target Pathogens:

1. *Staphylococcus aureus*
2. *Pseudomonas aeruginosa*
3. *Escherichia coli*
4. *Candida albicans*

General Materials Required:

Selective media:

Mannitol Salt Agar (for *S. aureus*)

Cetrimide Agar (for *P. aeruginosa*)

MacConkey Agar (for *E. coli*)

Sabouraud Dextrose Agar + Chloramphenicol (for *C. albicans*)

Tryptic Soy Broth or Nutrient Broth

Incubators at 30–35°C and 20–25°C

Sterile pipettes, test tubes, and Petri dishes

Sample Enrichment & Detection Procedure:

1. Enrichment Step:

Add 1 g or 1 mL of the face mist into 10 mL sterile Tryptic Soy Broth.

Incubate at 30–35°C for 24–48 hours.

This allows any low levels of pathogens to multiply to detectable levels.

2. Selective Plating:

From the enriched broth, streak 1 loopful onto each of the following:

- *S.aureus* - Mannitol salt agar- incubation for 30–35°C for 24–48 hours- Yellow colonies
- *P.aeruginosa*- Cetrimide agar - 30–35°C for 24–48 hours- Greenish colonies
- *E.coli*- MacConkey agar - 30–35°C for 24–48 hours – Pink colonies
- *C.albicans* – Sabouraud dextrose agar+ antibiotic – 20-25°C for 48 to 72 hrs – Creamy smooth colonies

3. Confirmation (Optional but Recommended):

Gram Staining (e.g., *S. aureus* is Gram-positive cocci)

Catalase & Coagulase Tests (for *S. aureus*)

Oxidase Test (for *P. aeruginosa*)

API strips or PCR (for precise ID in R&D)

Result Interpretation:

All 4 pathogens must be absent in 1 g or 1 mL sample.

Even a single colony indicates failure of microbial safety.



FIG :-Before incubation (Mannitol salt agar for pathogen)



FIG :-After incubation (Mannitol salt agar for pathogen)

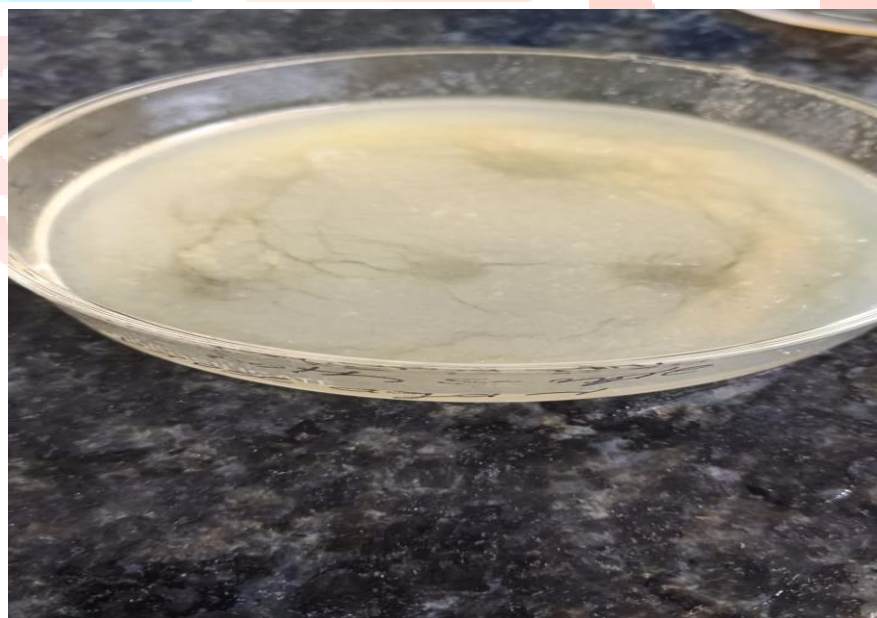


FIG :- Before incubation (Sabouraud dextrose agar for Pathogen)



FIG :- After incubation (sabouraud dextrose agar for Pathogen)

Pathogen Detection:

Selective media were used to identify *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*.

Results:-No presence of specific pathogens such as *E. coli*, *S. aureus*, *P. aeruginosa*, or *Candida albicans*.

The formulation demonstrated excellent microbiological safety. The absence of pathogens, indicates that the face mist is safe for topical application and complies with standard microbiological limits for cosmetic preparations.

RESULT

- 1 Colour :- Light yellow to pale green
- 2 Odour :- Fresh herbal citrus aroma
- 3 Texture :-Light non sticky
- 4 Sprayability Test:- Uniform coverage without clogging
- 5 pH Test :- pH is 5.04
- 6 Viscosity Test 12.36cp
- 7 Stability Test:-Stable at room temperature to 40°C and in refrigerator ($4 \pm 2^\circ\text{C}$)for 3 to 4 months
- 8 Irritation Test :-No redness ,rash and irritation was observed

- 9 Microbial load Test :- No visible bacterial colonies were observed on the Nutrient Agar plates.-Indicates satisfactory preservation and hygienic processing
No fungal or mold growth was seen on SDA plates during the incubation period.
- 10 Pathogens Specific Test :-No presence of specific Pathogen

Conclusion

The herbal face mist developed is safe for daily use on combination skin. With ethanol under 8%, it maintains antimicrobial properties while avoiding dryness. Plant actives such as quercetin, glycyrrhizin, and citral provide additional therapeutic benefits. Variants offer flexibility in formulation based on seasonal or skin-specific needs.

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