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Research Article

Formulation and Evaluation of Polyherbal Anti-Acne Face Wash Containing *Annona Squamosa* and *Clitoria ternatea* Extracts

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ABSTRACT

This study aimed to formulate and evaluate a herbal anti-acne face wash using extracts of *Annona squamosa* and *Clitoria ternatea*, known for their antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties. The extracts were prepared by maceration and incorporated into a gel-based face wash formulation. The prepared formulation showed good physicochemical properties such as suitable pH, smooth texture, good spreadability, stability, and no skin irritation. Phytochemical screening confirmed the presence of beneficial compounds like flavonoids, alkaloids, tannins, and saponins. Overall, the formulated herbal face wash was found to be safe, stable, and effective for the management of acne-prone skin.

INTRODUCTION

Annona squamosa (Custard Apple or "Sitaphal") is highly valued in pharmaceutical research for its potent **antimicrobial** and **wound-healing** properties, making it an excellent candidate for topical formulations. Its extracts contain bioactive compounds like acetogenins, flavonoids, and alkaloids that significantly enhance tissue repair, collagen synthesis, and epithelialization. Beyond its dermatological applications, it is traditionally recognized for its **anti-inflammatory**, **antioxidant**, and **antidiabetic** potential.(1)

Clitoria ternatea is primarily valued in pharmaceutical formulations for its potent **antioxidant** and **anti-inflammatory** properties, which accelerate wound

healing and tissue repair. It also serves as an effective **antimicrobial** agent for skin protection and acts as a natural **pH indicator**. Beyond topical uses, it is traditionally recognized for its **nootropic** (memory-enhancing) and **anxiolytic** (stress-relieving) effects.(2)

Acne vulgaris is a chronic inflammatory skin condition that primarily affects the pilosebaceous units, leading to the formation of comedones, papules, pustules, nodules, and, in severe cases, cysts. It is one of the most common dermatological disorders, impacting millions of individuals worldwide, particularly adolescents and young adults. The condition arises due to a combination of factors, including excessive sebum production, abnormal keratinization, bacterial colonization, and immune-mediated inflammation. Additionally, genetic

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predisposition, hormonal fluctuations, environmental factors, and dietary habits contribute to acne pathogenesis.

The anti acne face wash was designed to be non-greasy, easily absorbable, and cosmetically elegant to ensure patient compliance. To assess the efficacy and safety of the developed formulation, various parameters were evaluated, including physicochemical properties, stability, pH compatibility with human skin, antimicrobial activity, and anti-inflammatory potential. Additionally, skin irritation tests were conducted to determine the formulation's suitability for long-term topical use. By harnessing the therapeutic benefits of natural plant extracts, this research seeks to contribute to the development of safe and effective herbal-based acne treatments, addressing the growing demand for eco-friendly and non-toxic dermatological solutions (3,4)

Herbal anti acne face wash:

An effective herbal anti-acne face wash is defined by its ability to cleanse the skin while delivering bioactive compounds that address the root causes of acne. Unlike synthetic cleansers that often rely on harsh surfactants like Sodium Lauryl Sulfate (SLS), herbal formulations utilize natural saponins and plant extracts to maintain the skin's delicate acid mantle. These products are characterized by their multimodal therapeutic action: they simultaneously act as antimicrobial agents against *C. acnes*, anti-inflammatory agents to reduce erythema (redness), and sebum regulators to prevent pore clogging. Furthermore, the absence of parabens and artificial fragrances reduces the risk of contact dermatitis, making these formulations particularly suitable for sensitive or "acne-prone" skin types.

An ideal herbal anti-acne face wash is characterized by a synergistic blend of botanical extracts that provide antimicrobial, anti-inflammatory, and antioxidant activities. Unlike synthetic alternatives, it aims to manage acne vulgaris by inhibiting the growth of *Cutibacterium acnes* and *Staphylococcus epidermidis* while simultaneously regulating sebum production and providing a keratolytic effect to prevent pore clogging. Physically, the formulation must exhibit a skin-compatible pH (5.5–6.5) to maintain the acid mantle, high spreadability for ease of application, and excellent washability to ensure

no greasy residue remains. Furthermore, it should act as a gentle exfoliant, removing dead skin cells and impurities without stripping the skin of essential moisture, thereby promoting tissue repair and reducing the risk of post-inflammatory hyperpigmentation.(5,6)

Advantages of Herbal Anti-Acne Face Wash (7)

- **Maintains Moisture Barrier:** Cleanses effectively without stripping the skin of essential oils, which is crucial for combination skin.
- **Natural Antimicrobials:** Ingredients like Neem and Tea Tree naturally combat acne-causing bacteria.
- **Reduces Inflammation:** Components such as Turmeric and Aloe Vera help calm redness and soothe irritated breakouts.
- **Lower Sensitivity Risk:** Generally free from harsh sulfates, synthetic fragrances, and parabens, reducing the likelihood of allergic reactions or irritation.
- **Balanced Sebum Control:** Targets excess oil in the T-zone while remaining gentle on drier areas of the face.
- **Provides Antioxidant Support:** Naturally rich in nutrients that promote longterm skin health and healing rather than just acting as a temporary treatment.

Disadvantages of Herbal Anti-Acne Face Wash (8,9)

- **Slower Results:** Typically take longer to show visible improvements compared to clinical-strength chemical actives (e.g., salicylic acid, benzoyl peroxide).
- **Allergic Potential:** "Natural" does not mean non-allergenic. Plant extracts and essential oils are common triggers for contact dermatitis and skin irritation.
- **Limited Efficacy for Severe Acne:** Often insufficient for treating severe, cystic, or hormonal acne that requires medically proven, high-penetration ingredients.
- **Stability and Shelf Life:** Without strong synthetic preservatives, herbal formulations may degrade



faster, potentially reducing potency over time or increasing the risk of bacterial contamination.

- **Lack of Standardization:** The concentration and efficacy of active ingredients can vary significantly between batches based on the sourcing and processing of plant materials.
- **"Greenwashing" Risks:** The term "herbal" is not strictly regulated, leading to products that may market themselves as natural while still containing harsh or questionable synthetic ingredients.

2. AIM AND OBJECTIVES

Aim

To design, formulate, and evaluate a stable and effective herbal anti-acne face wash utilizing natural plant extracts with significant antimicrobial and anti-inflammatory properties.

Objectives (10)

- To Identify and select plant materials (herbal extracts) with scientifically validated anti-acne, anti-inflammatory, and antioxidant activities.
- To Extract active phytochemicals using appropriate extraction techniques and standardize the extracts to ensure consistent quality.
- To Develop a suitable dosage form (e.g., gel or liquid wash) and optimize the concentration of excipients (surfactants, humectants, preservatives) to ensure ingredient compatibility.
- To Conduct comprehensive testing on the formulated product, including:
- To Evaluate the *in-vitro* efficacy of the formulation against common acne-causing bacteria (e.g., *Cutibacterium acnes*, *Staphylococcus epidermidis*).
- To Perform accelerated stability studies to determine the shelf-life and ensure the integrity of the formulation under various environmental conditions.

3. NEED OF WORK

Need of work :(11,12)

The "need of work" (or rationale) for developing an herbal face wash centers on addressing the limitations of conventional, synthetic anti-acne treatments while catering to the growing consumer demand for safer, sustainable alternatives. While synthetic agents—such as clindamycin or benzoyl peroxide—are effective at combating acne-causing bacteria like *Cutibacterium acnes*, their prolonged use is often associated with adverse effects, including skin irritation, dryness, disruption of the natural skin barrier, and the increasing global concern of bacterial resistance. Furthermore, modern consumers are becoming more conscious of the long-term impact of chemical-laden products and are actively seeking biocompatible, "green" cosmetic solutions. By utilizing potent, naturally occurring phytoconstituents—such as those found in *Clitoria ternatea* and *Annonasquamosa*—pharmaceutical researchers can develop formulations that offer a synergistic approach, providing antimicrobial, anti-inflammatory, and antioxidant benefits. Consequently, the development of an herbal face wash is not merely a cosmetic endeavor but a therapeutic one, aiming to provide a safe, patient-compliant, and effective alternative that maintains skin health without the toxicity or sensitivity issues commonly associated with modern chemical entities.

Importance of antiacne facewash:(13)

The shift toward herbal-based anti-acne face washes in dermatological treatment is driven by the critical need to balance therapeutic efficacy with long-term skin health. Unlike many synthetic chemical agents—which, while potent, often compromise the skin barrier or induce bacterial resistance through single-target mechanisms—herbal formulations offer a **multifaceted, synergistic approach** to acne management. By integrating phytoconstituents known for their concurrent **antimicrobial, antiinflammatory, and antioxidant properties**, these washes effectively mitigate the key factors in acne pathophysiology—such as *Cutibacterium acnes* proliferation and follicular hyperkeratinization—without causing the excessive dryness or contact dermatitis frequently associated with



conventional medications. Furthermore, their superior safety profile allows for consistent, daily use, which is essential for successful acne management, thereby improving patient compliance and supporting the restoration of the skin's natural, healthy homeostasis while addressing the root causes of the condition rather than merely treating the symptoms.

Role of *Annona squamosa* in antiacne treatment:

Annona squamosa, commonly known as the custard apple, plays a significant role in anti-acne treatment primarily due to the diverse array of bioactive phytochemicals present in its leaves and extracts, such as annonaceousacetogenins, flavonoids, alkaloids, and terpenoids. Its effectiveness against acne is largely attributed to its potent **antimicrobial activity**, which disrupts the cellular integrity and metabolic pathways of acne-causing pathogens—specifically *Cutibacterium acnes* and *Staphylococcus epidermidis*—thereby inhibiting bacterial proliferation within the pilosebaceous unit. Furthermore, *Annona squamosa* exhibits substantial **anti-inflammatory and antioxidant properties**, which are critical for reducing the redness (erythema), swelling, and oxidative stress associated with inflammatory acne lesions. By suppressing pro-inflammatory mediators and neutralizing free radicals that exacerbate tissue damage, the plant extract aids in the resolution of acne breakouts and promotes skin healing, making it a highly promising natural candidate for incorporation into topical formulations that seek to restore skin homeostasis while avoiding the resistance issues associated with conventional

synthetic antibiotics.(14,15) **Role of *Clitoria ternatea* in antiacne face wash:**

Clitoria ternatea (Butterfly Pea) serves as a potent bioactive agent due to its rich phytoconstituent profile, primarily comprising flavonoids, anthocyanins (such as ternatins), and triterpenoids. Its role in acne treatment is largely attributed to its **significant anti-inflammatory and antioxidant activities**, which directly address the pathophysiology of acne—specifically the oxidative stress and inflammatory response triggered by *Cutibacterium acnes* and environmental factors. By inhibiting the activity of pro-inflammatory enzymes and cytokines, the extract helps reduce the redness, swelling, and irritation characteristic of inflamed acne lesions. Furthermore, its ability to scavenge free radicals and inhibit lipid peroxidation protects skin cells from oxidative damage, promoting a more stable environment for skin healing and repair.

When incorporated into a topical face wash, these multifaceted properties not only provide a soothing effect on existing breakouts but also contribute to long-term skin health by maintaining the structural integrity of the dermal matrix.(16)

4. PLANT PROFILE

4.1 PLANT PROFILE OF CLITORIA TERNATEA

Clitoria ternatea, belonging to the family Fabaceae, are among the largest and most diverse families of flowering plants, encompassing over 765 genera and more than 20,000 species worldwide[10]

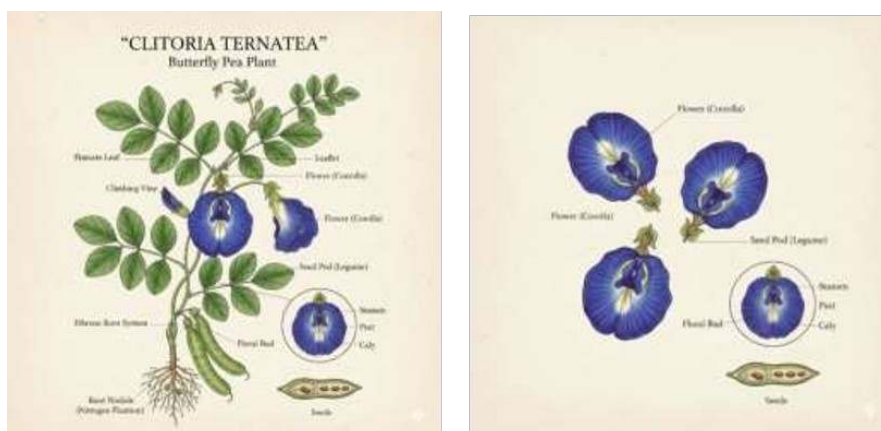


Figure 1: *Clitoria Ternatea*

Botanical classification:**Table 1: Botanical Classification:**

Rank	Classification
Kingdom	Plantae
Phylum	Tracheophyta/magnoliophylum
Class	Magnoliopsida/equisetopsida
Family	Fabaceae(leguminasae)
Genus	Clitoria
Species	Clitoria ternatea linn

Common Names[11]**Table 2: Common Names of *Clitoria Ternatea***

Language	Common Names
Hindi, Bengali, Oriya	अपराजिता or or
Gujarat	Koyala, Bismar, and Garani
Kannada	Billisaiuga, Satugadagida
Telugu	Dintana, Gilarnika, Neela-ghentana, Sankhupuvvu
Tamil	Kakkanam, Kakatan, Kavachi, Kuruvilai
Punjab	Dhanattar
Rajasthan	Koyalri, Titlimatar
English	Blue pea vine, mussel-shell climber, butterfly pea, and pigeon wings

Morphological characteristics:

The pea blossoms are about 4 cm long and 3 cm wide, with five petals. A banner with two wings, two keels, and a bright yellow insignia in the middle. The pea plant is one example. This legume climbing plant has incredibly thin leaves that are 1.5–3.5 cm and 2.5–5 cm in length. Every single width. This plant is evergreen and has fibrous roots. Nitrogen can be fixed by its massive nodules for use by other plants. Rhizobia are bacteria. Black seeds are used by the perennial plant *Clitoria ternatea* to reproduce. The colourful pods range in length from 7 to 11 cm. The culinary industry uses the root and leaves to create herbal and medicinal beverages. Teaching. The butterfly peas are the most popular in world

This plant is a hardy climber that adapts well to warm climates and can grow in a variety of soil types. It has nitrogen-fixing ability (being a legume), which

improves soil fertility. Its special features include bright blue flowers rich in anthocyanins, along with flavonoids and tannins that give it strong antioxidant, anti-inflammatory, and medicinal properties.

These compounds contribute to its use in wound healing and herbal formulations.[12,13]

Chemical constituents [14]

Flavonoids: These are plant antioxidants that help reduce inflammation and protect cells from oxidative damage. They also support tissue repair and improve wound healing.

Anthocyanins: Natural pigments responsible for blue/purple colour with strong antioxidant activity. They help in reducing oxidative stress and promote skin regeneration.

Alkaloids: Bioactive compounds with antimicrobial and analgesic properties. They help prevent infection and may reduce pain at the wound site.

Tannins: Polyphenolic compounds with astringent and antimicrobial effects. They help in wound contraction, reduce bleeding, and protect against infections.

Pharmacological activity

- Antioxidant activity: Protects cells from oxidative stress and damage
- Anti-inflammatory: Reduces swelling and inflammation
- Antimicrobial: Inhibits growth of bacteria and fungi
- wound healing: Promotes tissue repair and regeneration[15]

4.2 Plant profile of *Annona Squamosa*

Annona squamosa is a small tropical fruit-bearing tree belonging to the family Annonaceae. It is commonly known as custard apple or sitaphal and is widely cultivated in India and other warm regions of the world. The plant is valued for its sweet, creamy fruit as well as its medicinal properties. Different parts of the plant, including leaves, bark, seeds, and roots, are used in traditional medicine. It contains various bioactive compounds such as flavonoids, alkaloids, and tannins, which contribute to its therapeutic effects. *Annona squamosa* is known for its antioxidant, antimicrobial,



and anti-inflammatory activities. Due to these properties, it is widely studied for its potential in wound healing and other pharmaceutical applications.[16]

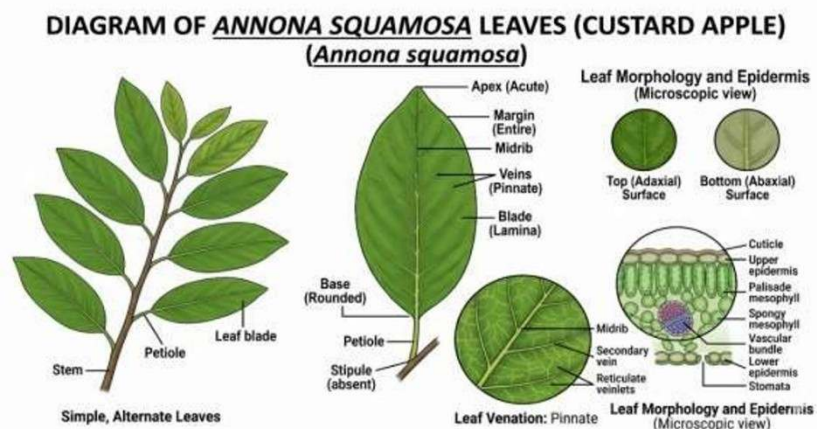


Figure 2: Anonna Squamosa

Table 3: Taxonomic Classification of *Annona Squamosa*

Level	Taxonomic Rank	Scientific Name	Common Name / Description
I	Kingdom	Plantae	Plants
II	Subkingdom	Tracheobionta	Vascular plants
III	Super division	Spermatophyta	Seed plants
IV	Division	Magnoliophyta	Flowering plants
V	Class	Magnoliopsida	Dicotyledons
VI	Subclass	Magnoliidae	—
VII	Order	Magnoliales	—
VIII	Family	Annonaceae	Custard apple family
IX	Genus	Annona	—
X	Species	Annona squamosa	Sugar apple [3,4]

***Regional Name:**

Table 4: Regional Names of *Annona Squamosa*

Language	Name(s)
English	Custard Apple, Sugar Apple, Sweet-sop
Hindi	सीताफल(Sitaphal)
Kannada	(Sitaphala)
Malayalam	(Sitaphalam), (Attachaka)
Sanskrit	सीताफलम् (Sitaphalam)
Tamil	(Sitaaphalam), (Atta)
Telugu	(Sitappalam)[3,4]

Morphological characteristics:

The pea blossoms are about 4 cm long and 3 cm wide, with five petals. A banner with two wings, two keels, and a bright yellow insignia in the middle. The pea

plant is one example. This legume climbing plant has incredibly thin leaves that are 1.5–3.5 cm and 2.5–5 cm in length. Every single width. This plant is evergreen and has fibrous roots. Nitrogen can be fixed by its massive nodules for use by other plants. Rhizobia are bacteria. Black seeds are used by the perennial plant *Clitoria ternatea* to reproduce. The colourful pods range in length from 7 to 11 cm. The culinary industry uses the root and leaves to create herbal and medicinal beverages. Teaching. The butterfly peas are the most popular in world.

This plant is a hardy climber that adapts well to warm climates and can grow in a variety of soil types. It has nitrogen-fixing ability (being a legume), which improves soil fertility.



Its special features include bright blue flowers rich in anthocyanins, along with flavonoids and tannins that give it strong antioxidant, anti-inflammatory, and medicinal properties.

These compounds contribute to its use in wound healing and herbal formulations.(45)

Chemical constituents

Flavonoids: These are plant antioxidants that help reduce inflammation and protect cells from oxidative damage. They also support tissue repair and improve wound healing.

Anthocyanins: Natural pigments responsible for blue/purple colour with strong antioxidant activity. They help in reducing oxidative stress and promote skin regeneration.

Alkaloids: Bioactive compounds with antimicrobial and analgesic properties. They help prevent infection and may reduce pain at the wound site.

Tannins: Polyphenolic compounds with astringent and antimicrobial effects. They help in wound contraction, reduce bleeding, and protect against infections.(46)

Pharmacological activity

- **Antioxidant activity:** Protects cells from oxidative stress and damage
- **Anti-inflammatory:** Reduces swelling and inflammation
- **Antimicrobial:** Inhibits growth of bacteria and fungi
- **wound healing:** Promotes tissue repair and regeneration (47)

Chapter 5 Material and Equipment's

5. MATERIAL AND EQUIPMENTS

Table 5: Material and Equipment

Sr. No.	Chemicals	Manufacturer
1	Ethanol	-----
2	Carbopol 934	THERMOSILE FINE CHEM INDUSTRIES
3	Propylene glycol	THERMOSILE FINE CHEM INDUSTRIES
5	Propyl paraben	MOLYCHEM
6	Triethanolamine	LOBA CHEM PVT.LTD
7	Sodium lauryl sulphate	THERMOSILE FINE CHEM INDUSTRIES
8	Glycerin	THERMOSILE FINE CHEM INDUSTRIES
9	Rose water	THERMOSILE FINE CHEM INDUSTRIES

Table 6: Instruments and Manufacturer

Sr. No.	Instruments	Manufacturer
1	Electronic analytical weighing balance	EQUIP-TRONICS
2	Electrical water bath	LABLINE
3	pH meter	EQUIP-TRONICS
4	Brookfield Viscometer (LVDV-60)	LABMAN
5	Heating mantel	LABLINE



EXPERIMENTAL METHODS

COLLECTION OF PLANTS

The development of a clitoria ternata and annova squamosa Extract facewash for antiacne Treatment requires the collection of high-quality plant materials and pharmaceutical excipients. The selection and procurement of these materials play a crucial role in ensuring the efficacy, safety, and stability of the final facewash formulation.

Collection of Plant Materials (clitoria ternatea Flowers and anova squamosa leaves)

Plant materials was systematically collected to ensure the highest concentration of bioactive compounds. (22)

Clitoria ternatea flowers:

- Medicinal clitoria ternatea was collected from natural habitats, botanical gardens, or authorized herbal farms in the Kolhapur district.
- Preference was given to clitoria ternatea species known for their antimicrobial, antioxidant, and skin-soothing properties.
- Flowers was hand-harvested early in the morning to retain maximum bioactive content.
- Collected flowers was washed, shade-dried, and stored properly to prevent degradation. (23)

Annona squamosal leaves:

- Selected mature, fully developed green leaves. Avoid young buds (low active content) and yellow, senescent leaves (degraded metabolites).
- Collected in the early morning (before the sun is fully up). This minimizes transpiration and degradation of heat-sensitive alkaloids and acetogenins.
- Picked only disease-free, clean, and intact leaves. Collected flowers was washed, shade-dried, and stored properly to prevent degradation (24)

EXTRACTION OF PLANT MATERIALS

The maceration extraction technique was used to obtain concentrated bioactive compounds from both clitoria ternatea and annona squamosal facewash preparation. This method was widely used due to its simplicity, cost-effectiveness, and ability to preserve thermo labile

compounds while efficiently extracting flavonoids, alkaloids, terpenoids, tannins and phenolic compounds. (25)

Procedure for Maceration Extraction

1. Preparation of extract

The collected clitoria ternatea flowers and anova squamosa leaves were dried under shade conditions to preserve the phytochemical integrity of the active compounds. Once dried, the plant materials were finely ground using a mechanical grinder to increase surface area, thereby improving the efficiency of extraction. The powdered material was accurately weighed for extraction.

2. Solvent Selection

Choosing an appropriate solvent is crucial for efficient extraction of bioactive compounds. Ethanol (70%) was used as they effectively extract flavonoids, alkaloids, terpenoids, and phenolic compounds—essential for anti acnetreatment. A solvent-to-sample ratio of 10:1 (v/w) was maintained to ensure optimal extraction

3. Maceration Process

The powdered plant materials were soaked in the selected solvent inside a closed glass container or maceration vessel. The mixture was agitated occasionally to improve solvent penetration and diffusion of bioactive compounds. The maceration process was carried out at room temperature for 48-72 hours to ensure maximum extraction of phytochemicals.

4.Filtration and Solvent Recovery

After maceration, the extract was filtered using Whitman filter paper or a Buchner funnel to remove solid residues. The solvent was then evaporated under reduced pressure using a rotary evaporator, yielding a concentrated extract.

5.Drying and Storage of Extracts

The obtained extracts were air-dried or freeze-dried to form a semi-solid or powdered form, ensuring long-term stability. Extracts were stored in amber glass vials under controlled temperature conditions to prevent degradation of bioactive compounds.



This maceration-based extraction ensures high bioavailability of *clitoria ternatea* and *anona squamosa* extracts, making them suitable for incorporation into the facewash formulation for anti acne treatment. (26)

Procurement of excipient

Pharmaceutical-grade excipients were procured from certified suppliers to ensure the stability, texture, absorption, and effectiveness of the facewash.

These excipients such as Carpool 934, Glycerine, Methylparaben, Triethanolamine (TEA), Distilled Water play a crucial role in maintaining the facewash's structural integrity, bioavailability and skin compatibility. (27)

Table 7: Excipient and Their Function

Excipient	Function in Gel Formulation
Carbopol 934	Gelling agent for viscosity and stability
Glycerin	Humectant to retain moisture
Methylparaben	Preservatives to prevent microbial growth
Triethanolamine (TEA)	pH adjuster for skin compatibility
Rose Water	Base for the gel formulation

PRELIMINARY INVESTIGATION

PHYTOCHEMICAL

After extracting bioactive compounds from *clitoria ternatea* flowers and *anona squamosa* leaves, a preliminary phytochemical screening was conducted to identify the presence of key secondary metabolites responsible for their therapeutic properties. Various qualitative tests are used to detect different classes of phytochemicals

1. Detection of Alkaloids

Both plants are known to contain alkaloidal compounds.

- Mayer's Test: Add a few drops of Mayer's reagent (Potassium Mercuric Iodide) to the extract. A cream-colored precipitate indicates presence.(28)

2. Detection of Flavonoids

Highly relevant for the anthocyanins in *Clitoria ternatea*.

- Shinoda Test: Add a few magnesium turnings and concentrated Hydrochloric acid (HCl) dropwise to the extract. A pink, scarlet, or crimson red color appears within minutes.(29)

3. Detection of Phenolics and Tannins

- Ferric Chloride Test: Mix the extract with a few drops of 5% Ferric Chloride (FeCl_3) solution.
 - o Blue-black colour: Indicates hydrolysable tannins.
 - o Brownish-green colour: Indicates condensed tannins.
- Lead Acetate Test: Add a few drops of 10% lead acetate solution to the extract. A bulky white precipitate indicates phenolic.(30,31)

4. Detection of Saponins

- Froth Test: Dilute the extract with distilled water to 20ml and shake vigorously in a graduated cylinder for 15 minutes. A persistent layer of foam (1 cm or more) indicates saponins.(31,32)

5. Detection of Glycosides

- Leal's Test: Dissolve the extract in pyridine and add alkaline sodium nitroprusside solution. A blood-red colour indicates cardiac glycosides.
- Keller-Killiani Test: To the extract, add glacial acetic acid, one drop of FeCl_3 and concentrated H_2SO_4 . A brown ring at the interface indicates deoxy sugars (33,34)

PREFORMULATION STUDY

Preformulation studies are essential to ensure the development of a stable, effective, and safe facewash dosage form. This stage of development involves characterizing the physicochemical properties of the drug substances and assessing their interaction with various formulation components.

Goals of Preformulation Study

- To determine the necessary physicochemical parameters of a new drug substance.
- To establish any incompatibility with excipients in the formulation. (35)

EXPERIMENTAL DESIGN

Procedure for the facewash Formulation



The formulation of clitoratearnatia and an-nonasquamosa involves the preparation of a stable facewash base, the incorporation of bioactive plant extracts, and the adjustment of physicochemical properties to ensure optimal therapeutic efficacy.(36)

Phase A: Gel Preparation (The Base)

1. **Dispersing:** Slowly sprinkle the gelling agent (e.g., 1g Carbopol 940) into 50ml of distilled water while stirring constantly to avoid clump formation.
2. **Hydration:** Allow the mixture to hydrate for 1–2 hours (or overnight) until a clear, uniform dispersion is formed.

Phase B: Extract & Surfactant Incorporation

3. **Surfactant Addition:** Slowly add the surfactant (e.g., 10–15% SLES) to the hydrated gel base. Stir gently to avoid excessive foaming.
4. **Extract Addition:** Dissolve your weighed extracts (*Annonasquamosa* and *Clitoria ternatea*) in a small amount of water or propylene glycol. Add this into the gel base while stirring.

Phase C: Additives & Neutralization

5. **Humectants & Preservatives:** Add Glycerin (approx. 5%) and your chosen preservative.
6. **pH Adjustment (Critical):** While stirring, add Triethanolamine drop-

wise. Carbopol is acidic; adding Triethanolamine thickens the gel and brings the pH to a skin-friendly level then forms the mixture transform from a thin liquid to a thick, transparent gel.(37)

FORMULATION TABLE

The facewash is developed to topically deliver bioactive compounds from clitoria ternatea extract (anti-inflammatory) and annonasquamosa leaves extract (antiacne) using a biocompatible facewash base that ensures stability, controlled release, and user acceptability. Formulating a facewash incorporating clitoria ternatea extract and annonasquamosa extract involves both pharmaceutical science and cosmetic formulation theory. The goal was develop a stable, bioactive, and biocompatible fcwash with effective release of active compounds from the clitoria ternatea and annonasquamosa extracts. (38)

Table 8: Formulation Table

Category	Ingredient	Quantity (%)	Role in Formulation
Active 1	<i>Annona squamosa</i> Extract	2.0 – 5.0	Antimicrobial & Antiinflammatory
Active 2	<i>Clitoria ternatea</i> Extract	1.0 – 2.0	Antioxidant & Natural Pigment
Gelling Agent	Carbopol 940	1.0 – 1.5	Provides viscosity and gel structure
Surfactant	Sodium Lauryl Ether Sulfate (SLES)	10.0 – 15.0	Primary cleansing & foaming agent
Humectant	Glycerin / Propylene Glycol	5.0	Prevents skin dryness (Moisturizer)
Neutralizer	Triethanolamine (TEA)	q.s.*	Adjusts pH and thickens the Carbopol
Preservative	Methylparaben / Propylparaben	0.2	Prevents microbial growth
Vehicle	Rose Water	q.s. 100	Continuous phase / Solvent

EVALUATION OF THE FACEWASH

1. Organoleptic and Physicochemical Evaluation

These tests assess the "shelf appeal" and basic chemical safety of the formulation.

- **Color, Odor, and Appearance:** Visual inspection to ensure the facewash is clear or translucent (depending on the extract concentration) and lacks any unpleasant medicinal smell.



- **pH Determination:** Since the facewash is for acne-prone skin, the pH must be maintained between **5.5 and 6.5**. A digital pH meter is used to ensure it matches the skin's natural acid mantle to prevent irritation.
- **Viscosity:** Measured using a **Brookfield Viscometer**. This ensures the product had the right flow properties—thick enough to stay on the hand, but fluid enough to be squeezed from the tube.

2. Performance & Rheological Tests

These evaluations determine how the product behaves during actual use.

• Foam Ability and Stability:

- **Method:** The cylinder shake method is used. 50 ml of the formulation is shaken in a 250 ml graduated cylinder.
- **Measurement:** The initial foam volume is recorded, and the stability is checked after 5, 10, and 15 minutes.
- **Spreadability:** Two glass slides are used to measure the "spread" of the gel under a specific weight. High spreadability indicates a better user experience and uniform application of the *Annonasquamosa* and *Clitoria ternatea* actives.
- **Washability:** Applied to the skin and rinsed with tap water. It should leave no greasy residue or

"slick" feeling, which is essential for acne-prone skin.

3. Stability Testing

This ensures the herbal extracts do not degrade or separate over time.

- **Accelerated Stability Studies:** Samples are placed in a stability chamber at **40°C ± 2°C / 75% ± 5% RH**.

4. Safety & Irritancy Studies

Even "natural" products can cause reactions.

- **Skin Irritancy Test (Patch Test):** A small amount of the facewash is applied to a 1 cm of the skin (often the dorsal surface of the forearm).
- **Observation:** The site is observed for 24 to 48 hours for signs of erythema (redness), edema (swelling), or itching. A "Non-Irritant" result is mandatory for a facewash.(39,40,41)

RESULT

The formulated *clitoria ternatea* and *annona squamosa* facewash was subjected to various physicochemical, antioxidant, antimicrobial, and stability evaluations to determine its suitability as a natural wound healing formulation. The results obtained from different evaluation parameters are discussed in detail below.

Table 9: Extractive Values of *Clitoria Ternatia* and *Annona Squamosa*

Sample	Extraction method	Solvent used	Wt. of sample	Extraction yield (%w/w)
Clitoria Ternatea	Maceration	Ethanol	10gm	10 %w/w
Annona Squamosa	Maceration	Ethanol	10gm	10 %w/w

a) Organoleptic Evaluation

The organoleptic properties of the facewash, including colour, odor, texture, and appearance, were evaluated to ensure aesthetic acceptability and user compliance.

Table 10: Parameters and Observation

Parameter	Observation
Color	Cobalt blue
Odor	Slightly herbal
Appearance	Uniform, no Separation

Parameter	Observation
Texture	Smooth, non-sticky



b) pH Measurement

The pH of the facewash is an essential factor in ensuring skin compatibility and preventing irritation. The measured pH of the facewash ranged between 4.5 and 5.5, which is close to the natural pH of human skin (4.75-5.75). This confirms that the formulation is mild, nonirritating, and safe for acne-prone skin, preventing excessive.

Dryness or disruption of the skin barrier.

pH of facewash: 5

c) Phytochemical Screenings

After extracting bioactive compounds from the *Annona Squamosa* and *Clitoria Ternatia*, a preliminary phytochemical screening is performed to detect the presence of key secondary metabolites responsible for their therapeutic properties. Various qualitative tests were carried out to identify these bioactive compounds.

Table 11: Phytochemical Screenings

Sr. No	Tests	Observation	Result
1	Mayer's Test	Formation of a creamy white precipitate	Presence of alkaloids was confirmed
2	Shinoda Test	Pink, scarlet, or crimson red colour appears within minutes.	Presence of flavonoids was confirmed
3	Lead Acetate Test	Bulky white precipitate	Presence of phenolics was confirmed
4	Froth Test	Formation of persistent froth	Presence of saponins was confirmed
5	Ferric Chloride Test	Formation of a dark blue/black colour	Presence of tannins was confirmed

d) Viscosity and Rheological Behaviour

Viscosity plays a vital role in the stability, application, and retention of topical formulations. The viscosity of

the facewash was found to be measured using a Brookfield viscometer. This is an ideal property for topical facewash, ensuring proper consistency and adherence to the skin.

Table 12: Viscosity and Rheological Behaviour

Batch	Viscosity (cps)	Rheological Behaviour	Observations
F1	3,000	Pseudo plastic	Thick, stable, easy to apply

e) Spread ability

Spreadability determines how easily a formulation spreads over the skin surface, ensuring effective application and absorption. The spreadability of the hydrogel was 12.45 cm/sec, indicating good application

properties. The facewash was neither too thick nor too fluid, allowing smooth application without excessive friction. The results confirm that the facewash spreads evenly, ensuring effective skin coverage and absorption.

Table 14: Spread Ability

Batch	Spread ability	Observations
F1	12.45	Good spread, minimal effort required

f) Stability Study

The stability of the formulation was evaluated under different storage conditions (4°C, 25°C, and 40°C) over

90 days. The facewash was tested for physical appearance, pH, viscosity, and microbial contamination.

Table 15: Stability Study

Parameter	Initial	30 Days	60 Days
Colour	Cobalt blue	No Change	No Change



Parameter	Initial	30 Days	60 Days
pH	5.0	5.4	5.4
Consistency	Moderate	Slightly Loose	Moderate

g) Skin Irritation Test

The absence of adverse effects suggests that the face-wash is well-tolerated and suitable for application on sensitive skin

Table 16: Skin Irritation Study

Batch	Irritation Observed	Result
F1	None	Safe

CONCLUSION

The present study successfully developed and evaluated an *Annona squamosa* and *clitoria ternatea* extract facewash for the treatment of acne. The combination of *Annona squamosa* and *Clitoria ternatea* represents a promising phyto-cosmeceutical approach. The resulting facewash serves as more than a simple cleanser; it acts as a therapeutic agent capable of neutralizing free radicals, inhibiting microbial growth, and promoting skin rejuvenation. The formulation acts as a multi-targeted anti-acne agent. promotes tissue repair, significantly reducing the risk of post-acne scarring. Overall, this study highlights the potential of herbal-based facewash formulations as a safe, effective, and natural alternative for acne management

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