Formulation and Comprehensive Evaluation of a Polyherbal Anti-Acne Face Pack Using Medicinal Plant Extracts for Topical Skin Care Applications

Pradnya Pradeep Wadekar[1], Priyanka R. Kalkutagi[2], Anil A. Danane[2], Javeed Manure[2], Pramod B. Patil[3], Akshata S. Patil[4], Tejashree Sourabh Khamkar[4], Kajal Manjunath chougule[4]

[1] Department of Pharmacognosy, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India
[2] Department of Pharmaceutical Quality Assurance, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India
[3] Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, India
[4] Department of Pharmaceutical chemistry, Ashokrao Mane college of pharmacy peth vadgaon

PPW: wadekar_p@yahoo.co.in, **PRK:** priyankkalkutagi@gmail.com, **AAD:** aad@abcpsangli.edu.in, **JM:** javeedmanure.1986@gmail.com, **PBP:** pramodbpatil24@gmail.com, **ASP:** patilakshata394@gmail.com, **TSK:** tejshreekhade22@gmail.com, **KMC:** kajalchougule3072@gmail.com

Corresponding Author: Dr. Pradnya Pradeep Wadekar
Department of Pharmacognosy, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India
E-mail: wadekar_p@yahoo.co.in

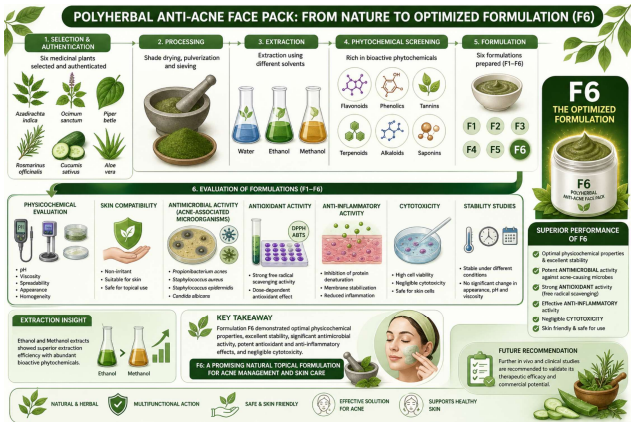
Abstract

Acne vulgaris is a common chronic inflammatory skin disorder that affects a large proportion of adolescents and adults worldwide. Conventional therapies are often associated with adverse effects, poor patient compliance, and the emergence of antimicrobial resistance, highlighting the need for safer and more effective alternatives. The present study aimed to formulate and evaluate a polyherbal anti-acne face pack containing *Azadirachta indica*, *Ocimum sanctum*, *Piper betle*, *Rosmarinus officinalis*, *Cucumis sativus*, and *Aloe vera*. The selected medicinal plants were authenticated, subjected to pharmacognostic evaluation, extracted using different solvents, and screened for phytochemical constituents. Six formulations (F1–F6) were prepared and evaluated for physicochemical characteristics, pH, viscosity, spreadability, skin compatibility, antimicrobial, antioxidant, anti-inflammatory, cytotoxicity, and stability. Ethanol and methanol extracts exhibited superior extraction efficiency with abundant bioactive phytochemicals, including flavonoids, phenolics, tannins, terpenoids, alkaloids, and saponins. Among the formulations, F6 demonstrated optimal physicochemical properties, excellent stability, significant antimicrobial activity against acne-associated microorganisms, potent antioxidant and anti-inflammatory effects, and negligible cytotoxicity. These findings suggest that the optimized polyherbal face pack is a promising natural topical formulation for acne management and skin care. Further in vivo and clinical studies are recommended to validate its therapeutic efficacy and commercial potential.

Keywords: Acne vulgaris, Polyherbal face pack, Medicinal plants, Antimicrobial activity, Antioxidant activity

How to cite this article: Wadekar PP, Kalkutagi PR, Danane AA, Manure J, Patil PB, Patil AS, Khamkar TS, Chougule KM. Formulation and Comprehensive Evaluation of a Polyherbal Anti-Acne Face Pack Using Medicinal Plant Extracts for Topical Skin Care Applications. Int J Drug Deliv Technol. 2026;16(69s):1215-1230. DOI: 10.25258/ijddt.16.69s.122

Graphical Abstract



Highlights

- A novel polyherbal anti-acne face pack containing six medicinal plants was successfully developed.

- Ethanol extracts exhibited the highest phytochemical richness and extraction efficiency.
- The optimized formulation demonstrated excellent physicochemical properties and skin compatibility.
- Significant antimicrobial, antioxidant, and anti-inflammatory activities supported its therapeutic potential.
- The developed formulation represents a safe, economical, and sustainable alternative to conventional anti-acne products.

1. Introduction

Acne vulgaris is one of the most prevalent chronic inflammatory disorders of the pilosebaceous unit, affecting approximately 80–90% of adolescents and a considerable proportion of adults worldwide. Although acne is generally regarded as a self-limiting condition, persistent or severe disease can result in permanent scarring, post-inflammatory hyperpigmentation, psychological distress, anxiety, depression, and reduced quality of life. The increasing prevalence of acne, particularly among young adults, has transformed it from a cosmetic concern into a significant dermatological and public health issue. The multifactorial pathogenesis of acne involves excessive sebum production, follicular hyperkeratinization, colonization by *Cutibacterium acnes* (formerly *Propionibacterium acnes*), and inflammatory responses mediated by cytokines and reactive oxygen species (ROS). These interconnected mechanisms create an environment conducive to comedone formation and inflammatory lesions, including papules, pustules, nodules, and cysts [1].

Current therapeutic approaches include topical retinoids, benzoyl peroxide, antibiotics, salicylic acid, azelaic acid, hormonal therapy, and systemic isotretinoin. While these treatments are clinically effective, their prolonged use is frequently associated with adverse effects such as erythema, skin irritation, peeling, photosensitivity, allergic reactions, dryness, and disruption of the skin barrier. Moreover, the widespread use of topical and systemic antibiotics has contributed to the alarming emergence of antimicrobial resistance among acne-associated microorganisms, thereby reducing treatment efficacy and necessitating the exploration of alternative therapeutic strategies. Consequently, there is increasing scientific interest in developing safer, multifunctional, plant-based topical formulations capable of simultaneously targeting microbial infection, inflammation, oxidative stress, and impaired skin healing [2].

Herbal medicines have served as an integral component of traditional healthcare systems for centuries and continue to attract considerable attention owing to their therapeutic efficacy, favorable safety profile, cost-effectiveness, and broad spectrum of pharmacological activities. Medicinal plants contain diverse bioactive phytochemicals, including flavonoids, phenolic acids, alkaloids, terpenoids, tannins, glycosides, saponins, and essential oils, many of which exhibit potent antimicrobial, antioxidant, anti-inflammatory, wound-healing, and immunomodulatory properties. Unlike synthetic drugs that often act through a single pharmacological pathway, herbal formulations provide synergistic therapeutic effects through multiple mechanisms, making them particularly suitable for managing multifactorial disorders such as acne vulgaris [3-4].

The global demand for herbal cosmetics and cosmeceuticals has increased substantially over the past decade due to growing consumer awareness regarding the potential adverse effects of synthetic ingredients. Herbal face packs have emerged as an attractive topical dosage form because they combine therapeutic efficacy with cosmetic benefits. These formulations help cleanse the skin, absorb excess sebum, remove impurities, reduce microbial load, soothe inflammation, improve skin texture, and enhance overall skin appearance. In contrast to creams and ointments, face packs generally exhibit superior patient acceptability owing to their non-greasy nature, ease of application, pleasant sensory characteristics, and minimal risk of occluding skin pores.

Selection of medicinal plants with complementary biological activities is a critical factor in the development of effective polyherbal dermatological formulations. *Azadirachta indica* (Neem) is widely recognized for its broad-spectrum antimicrobial, anti-inflammatory, antioxidant, and wound-healing activities, primarily attributed to phytoconstituents such as nimbidin, nimbolide, azadirachtin, quercetin, and various limonoids. Neem has demonstrated inhibitory activity against acne-associated microorganisms while reducing inflammatory cytokine production and oxidative stress [4-5].

Ocimum sanctum (Holy Basil or Tulsi) possesses remarkable antimicrobial, antioxidant, and immunomodulatory properties due to the presence of eugenol, ursolic acid, rosmarinic acid, flavonoids, and polyphenols. These compounds effectively suppress microbial proliferation while modulating inflammatory mediators involved in acne pathogenesis. Additionally, Tulsi contributes to improved skin healing through its antioxidant potential.

Piper betle (Betel leaf) has long been used in traditional medicine for the treatment of skin infections and inflammatory disorders. The plant contains phenolic compounds such as hydroxychavicol, chavibetol, eugenol, and various terpenoids that exhibit strong antibacterial,

antifungal, antioxidant, and anti-inflammatory activities. These phytochemicals contribute to reducing microbial colonization and minimizing oxidative damage associated with inflammatory acne lesions [6-8].

Rosmarinus officinalis (Rosemary) is a rich source of rosmarinic acid, carnosic acid, carnosol, and essential oils that provide potent antioxidant and antimicrobial effects. Rosemary extracts have demonstrated significant free radical scavenging activity while inhibiting inflammatory mediators responsible for tissue damage and delayed skin repair. Their incorporation into topical formulations may therefore enhance therapeutic outcomes in acne management.

Cucumis sativus (Cucumber) is extensively used in cosmetic preparations because of its soothing, cooling, moisturizing, and antioxidant properties. It contains flavonoids, vitamin C, phenolic compounds, minerals, and polysaccharides that help maintain skin hydration, reduce irritation, and improve overall skin condition. The addition of cucumber extract further enhances patient comfort during topical application [8-10].

Aloe vera has been extensively investigated for its moisturizing, wound-healing, anti-inflammatory, antimicrobial, and antioxidant activities. Bioactive constituents such as acemannan, aloin, aloe-emodin, vitamins, and polysaccharides contribute to accelerated tissue regeneration, maintenance of skin hydration, and reduction of inflammation. Aloe vera also improves the texture and spreadability of topical formulations, making it an ideal component of herbal dermatological products. **Figure 1.** Shows Global overview of acne vulgaris pathogenesis showing follicular hyperkeratinization, sebum overproduction, *Cutibacterium acnes* colonization, oxidative stress, and inflammation.

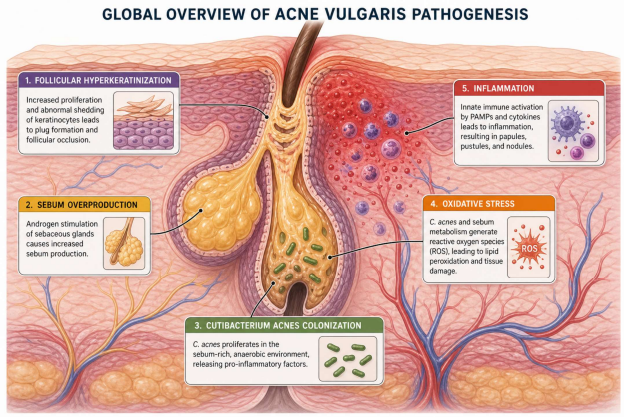


Figure 1. Shows Global overview of acne vulgaris pathogenesis

The rationale for combining these medicinal plants lies in their complementary mechanisms of action. The antimicrobial constituents reduce the growth of acne-associated microorganisms, antioxidant phytochemicals neutralize reactive oxygen species responsible for inflammatory damage, while anti-inflammatory compounds suppress cytokine-mediated skin inflammation. Simultaneously, wound-healing and moisturizing components promote tissue repair and restore the integrity of the skin barrier. Such a multifunctional approach is expected to provide greater therapeutic benefit than formulations based on individual plant extracts [11-12].

Although several herbal anti-acne products are commercially available, most contain a limited number of botanical ingredients and lack comprehensive scientific validation regarding pharmacognostic quality, phytochemical composition, physicochemical characteristics, antimicrobial efficacy, antioxidant potential, anti-inflammatory activity, cytotoxicity, and formulation stability. Furthermore, systematic investigations evaluating synergistic combinations of multiple medicinal plants remain relatively limited [13-15].

Therefore, the present study was designed to develop and comprehensively evaluate a novel polyherbal anti-acne face pack containing *Azadirachta indica*, *Ocimum sanctum*, *Piper betle*, *Rosmarinus officinalis*, *Cucumis sativus*, and *Aloe vera*. The selected medicinal plants were subjected to pharmacognostic evaluation, solvent extraction, and phytochemical screening prior to formulation development. The prepared formulations were characterized for physicochemical properties, skin compatibility, antimicrobial activity, antioxidant potential, anti-inflammatory activity, cytotoxicity, and stability to identify an optimized herbal face pack with potential application in acne management and dermatological skincare [16-18].

2. Materials and Methods

2.1 Materials

Fresh leaves of *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Piper betle* (Betel leaf), *Rosmarinus officinalis* (Rosemary), fresh fruits of *Cucumis sativus* (Cucumber), and mature *Aloe vera* leaves were collected from Sangli district, Maharashtra, India, during the appropriate harvesting season. The collected plant materials were authenticated by a qualified taxonomist, and

voucher specimens were deposited in the departmental herbarium for future reference.

Multani mitti, rose water, and glycerin were procured from certified local suppliers, while all analytical-grade solvents including ethanol, methanol, chloroform, ethyl acetate, and other laboratory reagents were purchased from Molychem Pvt. Ltd., Mumbai, India. All chemicals used throughout the study were of analytical reagent (AR) grade.

2.2 Authentication and Processing of Plant Materials

The collected plant materials were washed thoroughly with distilled water to remove adhering soil and foreign matter. Samples were shade dried at ambient temperature (25–30°C) for 7–10 days until constant weight was achieved. The dried materials were pulverized using a mechanical grinder and passed through sieve No. 60 to obtain uniform powder. The powdered samples were stored in airtight containers protected from moisture and light until further use.

2.3 Pharmacognostic Evaluation

The powdered crude drugs were subjected to standard pharmacognostic evaluation according to WHO guidelines. Moisture content was determined by loss on drying at 105°C until constant weight. Total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive value, and water-soluble extractive value were determined following standard pharmacopeial procedures.

Each experiment was carried out in triplicate, and the results were expressed as mean ± standard deviation [19-21].

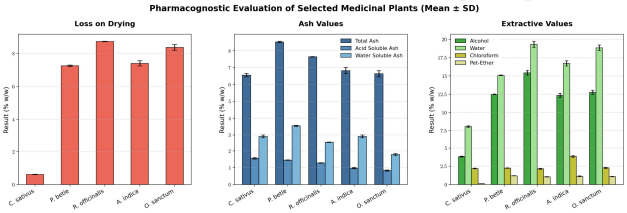


Figure 2: Flow diagram showing pharmacognostic evaluation of medicinal plants

2.4 Preparation of Plant Extracts

2.4.1 Preparation of Powder

The authenticated medicinal plants were shade dried and pulverized separately using a laboratory grinder.

2.4.2 Extraction

Approximately 50 g of each powdered plant material was extracted separately using Soxhlet extraction with water, ethanol, methanol, chloroform, and ethyl acetate.

Extraction was continued until the siphon tube became colourless.

The extracts were filtered through Whatman No.1 filter paper and concentrated under reduced pressure using a rotary evaporator.

The concentrated extracts were dried in a vacuum desiccator and stored at 4°C until further investigation [22-24].

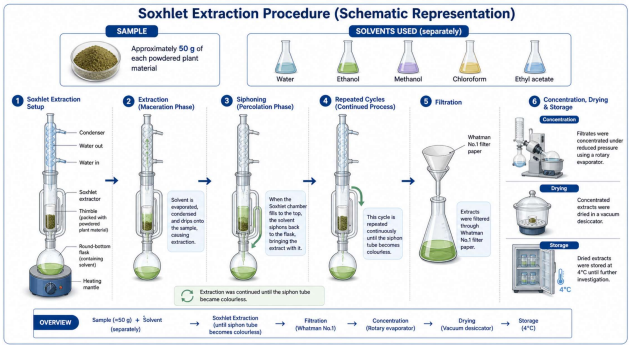


Figure 3: Schematic representation of Soxhlet extraction procedure

2.5 Determination of Percentage Yield

Extraction efficiency was determined using the following equation.

$$\text{Percentage Yield} = \frac{\text{Weight of dried extract}}{\text{Weight of crude drug}} \times 100$$

2.6 Preliminary Phytochemical Screening

The extracts were screened for major classes of phytochemicals using standard qualitative chemical tests.

The following constituents were evaluated:

- Alkaloids
- Flavonoids
- Tannins
- Phenolics
- Glycosides
- Terpenoids
- Steroids
- Saponins
- Proteins
- Carbohydrates
- Fixed oils
- Resins

The presence or absence of phytochemicals was recorded based on colour development or precipitate formation.

Table 1: Qualitative phytochemical tests employed

Phytochemical	Test Used
Alkaloids	Dragendorff's
Flavonoids	Alkaline reagent
Tannins	Ferric chloride
Phenols	Ferric chloride
Glycosides	Keller-Killiani
Steroids	Liebermann-Burchard
Terpenoids	Salkowski
Saponins	Foam test

2.7 Formulation of Polyherbal Face Pack

Six formulations (F1–F6) were prepared by varying the concentration of medicinal plant powders while maintaining constant amounts of Multani mitti, glycerin, and rose water.

The powdered herbal ingredients were mixed uniformly by geometric dilution. Rose water and glycerin were added gradually under continuous stirring to obtain a homogeneous paste. The prepared formulations were transferred into airtight containers and stored at room temperature until evaluation [25-27]..

Table 2: Composition of polyherbal face pack formulations (F1–F6)

Formulation Code	Multani Mitti (g)	Neem Powder (g)	Tulsi Powder (g)	Betel Leaf Extract (g)	Cucumber Extract (g)	Rosemary Extract (g)	Aloe Vera Gel (g)	Glycerine (ml)	Rose Water QS (ml)
F1	5	1	2	1	4	3	5	4	QS
F2	5	1	4	1	4	3	5	4	QS
F3	5	2	2	1	4	3	5	4	QS
F4	5	2	4	1	4	3	5	4	QS
F5	5	4	2	2	3	2	5	2	QS
F6	**5**	**4**	**4**	**2**	**3**	**2**	**5**	**2**	**QS**

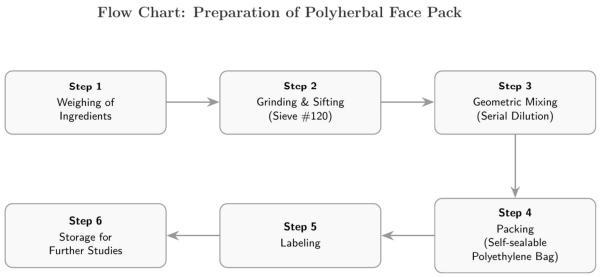


Figure 4: Flow chart illustrating preparation of polyherbal face pack

2.8 Evaluation of Prepared Formulations

The prepared formulations were evaluated for the following parameters.

2.8.1 Organoleptic Properties

Colour
Odour
Texture
Appearance
Homogeneity

2.8.2 pH

One gram of formulation was dispersed in 100 mL distilled water. The pH was measured using a calibrated digital pH meter.

2.8.3 Spreadability

Spreadability was determined using the parallel plate method.

The spreadability coefficient was calculated as

$$S = \frac{M \times L}{T}$$

where
M = weight tied to upper slide
L = length moved
T = time required

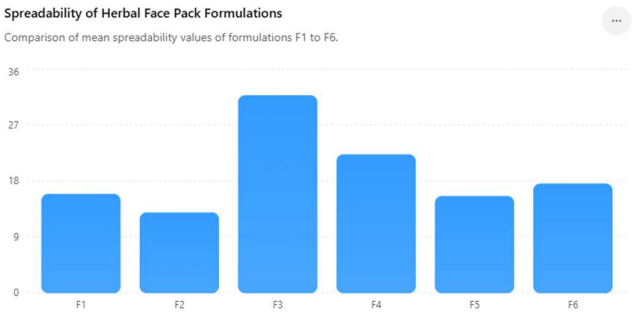


Figure 5: Spreadability test setup

2.8.4 Viscosity

Viscosity was measured using a Brookfield cone-and-plate rheometer at room temperature. Measurements were performed in triplicate.

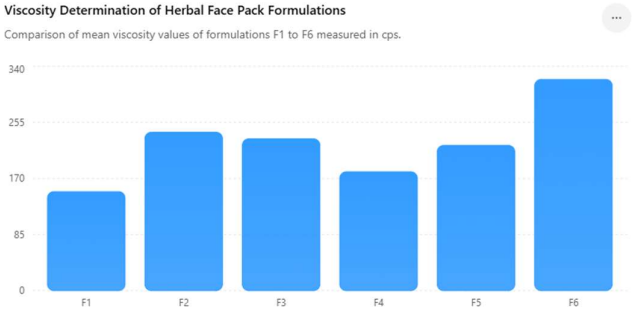


Figure 6: Brookfield rheometer used for viscosity determination

2.8.5 Skin Irritation Study

The prepared formulation was applied on the dorsal skin of healthy volunteers/experimental model (state according to your ethics approval). The treated area was observed for erythema, edema, itching, or irritation over 24 h.

2.9 Antimicrobial Activity

The antimicrobial activity was evaluated using the agar well diffusion method. Test microorganisms included

- *Staphylococcus aureus*
- *Escherichia coli*
- *Candida albicans*
- *Aspergillus niger*

Standard antibiotics served as positive controls, while the vehicle served as the negative control. Zones of inhibition were measured after incubation.

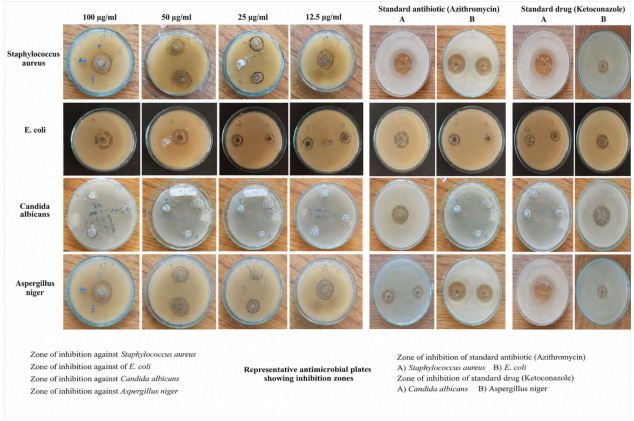


Figure 7: Representative antimicrobial plates showing inhibition zones

2.10 Antioxidant Activity

The antioxidant activity was evaluated by DPPH radical scavenging assay.

Different concentrations of the optimized formulation were incubated with DPPH solution for 30 min in the dark. Absorbance was measured at 517 nm.

Percentage inhibition was calculated as

$$\%\ Inhibition = \frac{A_0 - A_s}{A_0} \times 100$$

2.11 Anti-inflammatory Activity

The anti-inflammatory activity was determined using the inhibition of bovine serum albumin (BSA) denaturation assay. After incubation, absorbance was measured spectrophotometrically.

Percentage inhibition was calculated. Diclofenac sodium was used as the reference standard [28-32].

2.12 Cytotoxicity Study

Cytotoxicity was evaluated using the brine shrimp lethality assay. Brine shrimp nauplii were exposed to different concentrations of the optimized formulation for 24 h. Mortality percentage was recorded and LC_{50} values were calculated [33-34].

2.13 Stability Study

The optimized formulation was stored under accelerated conditions (40 ± 2°C/75 ± 5% RH) and room temperature for three months according to ICH guidelines. Samples were periodically evaluated for

- Appearance
- pH
- Spreadability
- Viscosity
- Odour
- Microbial growth [34-35].

Table 3: Parameters evaluated during stability studies

Parameter	Initial	After 30 Days	After 60 Days
Appearance	Smooth semisolid	No change	No change
Color	Light green	No change	No change
Odor	Pleasant	No change	No change
Texture	Smooth	Smooth	Smooth
pH	6.3	6.2	6.1
Spreadability (g.cm/sec)	17.5	17.2	16.9
Viscosity (cps)	320	318	315
Irritation	Nil	Nil	Nil

2.14 Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using GraphPad Prism (Version XX) or SPSS (Version XX). Differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1 Pharmacognostic Evaluation of Selected Medicinal Plants

The physicochemical characteristics of the selected medicinal plants were evaluated to establish their identity, purity, and suitability for formulation development. Parameters including loss on drying, total ash, acid-insoluble ash, water-soluble ash, and solvent extractive values were determined according to standard pharmacognostic procedures.

The results demonstrated acceptable moisture content in all plant materials, indicating adequate drying and reduced risk of microbial deterioration during storage. Total ash values were within pharmacopeial limits, suggesting minimal inorganic contamination, while low acid-insoluble ash values confirmed negligible siliceous impurities. Water- and alcohol-soluble extractive values varied among the selected plants, reflecting differences in their phytochemical composition and solvent affinity. Overall, the pharmacognostic findings confirmed the quality, purity, and authenticity of the crude plant materials used for formulation development.

Table 4. Pharmacognostic characteristics of selected medicinal plants. [4.1-4.5]

Table 4.1 Pharmacognostic Study of *Cucumis sativus L.*

Sr. No.	Parameter Studied	Result (mean + SD) % w/w
1.	Loss on drying	0.61 +0.02
Ash Value		
2.	Total Ash	6.55 +0.10
	Acid soluble Ash	1.56 +0.05
	Water soluble Ash	2.92 +0.08
Extractive values		
3.	Alcohol	3.83 +0.07
	Water	8.07 +0.12
	Chloroform	2.20 +0.06
	Pet-Ether	0.12 +0.01

Table 4.2 Pharmacognostic Study of *Piper betle L.*

Sr. No.	Parameter Studied	Result (mean + SD) % w/w
1.	Loss on drying	7.25 +0.05
Ash Value		
2.	Total Ash	8.52 +0.04
	Acid soluble Ash	1.45 +0.02
	Water soluble Ash	3.54 +0.03
Extractive values		
3.	Alcohol	12.48 +0.05
	Water	15.09 +0.05
	Chloroform	2.25 +0.02
	Pet-Ether	1.20 +0.02

Table 4.3 Pharmacognostic Study of *Rosmarinus officinalis L.*

Sr. No.	Parameter Studied	Result (mean + SD) % w/w
1.	Loss on drying	8.72 +0.01
Ash Value		
2.	Total Ash	7.64 +0.01
	Acid soluble Ash	1.28 +0.01
	Water soluble Ash	2.57 +0.01
Extractive values		
3.	Alcohol	15.42 +0.32
	Water	19.33 +0.41
	Chloroform	2.16 +0.07

	Pet-Ether	1.06 ±0.04

Table 4.4 Pharmacognostic Study of *Azadirachta indica*

Sr. No.	Parameter Studied	Result (mean ± SD) % w/w
1.	Loss on drying	7.39 ±0.16
Ash Value		
2.	Total Ash	6.82 ±0.19
	Acid soluble Ash	0.98 ±0.04
	Water soluble Ash	2.92 ±0.08
Extractive values		
3.	Alcohol	12.34 ±0.28
	Water	16.71 ± 0.36
	Chloroform	3.85 ± 0.11
	Pet-Ether	1.13 ±0.05

Table 4.5 Pharmacognostic Study of *Ocimum sanctum*

Sr. No.	Parameter Studied	Result (mean ± SD) % w/w
1.	Loss on drying	8.36 ±0.17
Ash Value		
2.	Total Ash	6.63 ±0.18
	Acid soluble Ash	0.83 ±0.03
	Water soluble Ash	1.78 ±0.06
Extractive values		
3.	Alcohol	12.75 ±0.29
	Water	18.86 ±0.38
	Chloroform	2.28 ±0.08
	Pet-Ether	1.09 ±0.04

3.2 Extraction Yield

Extraction efficiency varied considerably depending on both the solvent polarity and the botanical source. Polar solvents, particularly ethanol and methanol, produced higher extraction yields than water, chloroform, and ethyl acetate for most plant materials. The superior extraction efficiency of ethanol and methanol may be attributed to their greater ability to solubilize polar phytochemicals, including phenolics, flavonoids, glycosides, and tannins.

Among the evaluated medicinal plants, *Azadirachta indica*, *Piper betle*, and *Rosmarinus officinalis* exhibited comparatively higher extractive yields in ethanol and methanol, indicating their richness in bioactive constituents. These findings supported the selection of ethanolic extracts for subsequent formulation studies.

Table 5. Percentage extraction yield of medicinal plants using different solvents.

Solvent	Extraction Yield (% w/w)				
	Cucumis sativus L.	***Piper betle L.***	***Rosmarinus officinalis L.***	***Azadirachta indica***	***Ocimum sanctum***
Water	9.0%	8.5%	7.5%	7.8%	10.5%
Ethanol	14.5%	15.2%	13.1%	13.5%	12.90%
Methanol	13.8%	13.7%	12.95%	14.6%	13.85%
Chloroform	11.5%	11.0%	10.25%	10.5%	10.08%
Ethyl Acetate	8.0%	8.0%	9.08%	8.0%	8.8%

3.3 Preliminary Phytochemical Screening

Qualitative phytochemical analysis revealed the presence of several biologically active secondary metabolites in all selected medicinal plants. Flavonoids, phenolic compounds, tannins, terpenoids, alkaloids, saponins, glycosides, and steroids were detected with varying intensity depending on the extraction solvent.

Ethanol and methanol extracts consistently exhibited a broader phytochemical profile than aqueous and non-polar extracts. Strong positive reactions for phenolics, flavonoids, and terpenoids suggested substantial antioxidant and antimicrobial potential. The abundance of these phytochemicals supports the therapeutic rationale for combining the selected medicinal plants in a polyherbal anti-acne formulation.

Table 6: Preliminary phytochemical screening of medicinal plant extracts.

Phytochemical	Water Extract	Ethanol Extract	Methanol Extract	Chloroform Extract	Ethyl Acetate
Alkaloids	-	+	+	-	-
Flavonoids	++	++	++	-	-
Tannins	++	++	++	-	-
Saponins	++	++	++	-	-
Steroids	-	++	++	++	++
Terpenoids	+	++	++	++	++
Glycosides	+	+	+	-	-
Phenols	++	+++	+++	+	-
Carbohydrates	+++	++	++	-	-
Proteins & Amino Acids	+	+	+	-	-
Resins	-	-	-	-	-

Fats & Oils	-	-	-	+	++

3.4 Formulation Development

Six polyherbal face pack formulations (F1–F6) were successfully prepared using different proportions of herbal extracts and excipients. All formulations exhibited satisfactory appearance, uniform consistency, and pleasant herbal odor without visible particulate matter or phase separation.

The formulations were smooth, homogeneous, and easily washable, indicating good cosmetic acceptability. Among the prepared formulations, F6 demonstrated superior physical appearance and handling characteristics and was selected for further evaluation.

3.5 Organoleptic and Physicochemical Evaluation

All prepared formulations exhibited acceptable organoleptic characteristics with uniform color, pleasant odor, smooth texture, and good homogeneity. The measured pH values ranged within the physiological skin pH, suggesting compatibility with topical application and a low probability of skin irritation.

Viscosity measurements indicated desirable rheological behavior, providing adequate consistency for easy application while preventing excessive runoff. Similarly, spreadability testing demonstrated that the formulations could be uniformly distributed over the skin surface with minimal applied force, enhancing user compliance.

Among all formulations, F6 exhibited the most favorable balance between viscosity, spreadability, and pH, indicating optimal formulation characteristics.

3.6 Skin Compatibility Study

The skin irritation study demonstrated excellent dermal compatibility of the optimized formulation. No visible signs of erythema, edema, itching, burning sensation, or allergic reactions were observed throughout the observation period.

These findings suggest that the selected medicinal plant extracts and excipients are safe for topical application and suitable for long-term cosmetic use.

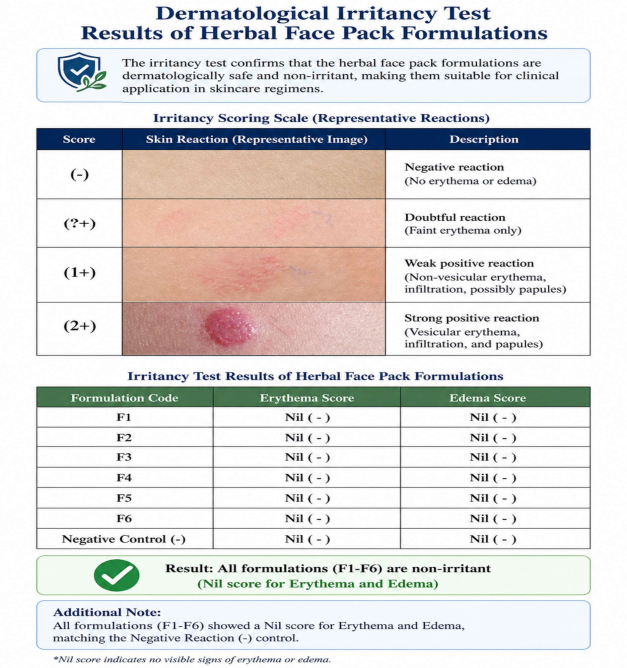


Figure 8: Irritancy Test of the Face Pack Formulation

3.7 Antimicrobial Activity

The antimicrobial activity of the developed formulations was evaluated against representative Gram-positive bacteria, Gram-negative bacteria, and fungal strains commonly associated with skin infections.

The optimized formulation (F6) produced the largest zones of inhibition against all tested microorganisms. Strong antibacterial activity was observed against *Staphylococcus aureus*, followed by *Escherichia coli*, whereas substantial antifungal activity was also demonstrated against *Candida albicans* and *Aspergillus niger*.

The enhanced antimicrobial activity may be attributed to the synergistic effects of flavonoids, phenolics, terpenoids, and essential oils present in the selected medicinal plants.

Table 7. Antimicrobial activity.

Microorganism	Zone of Inhibition (mm)					
	F1	F2	F3	F4	F5	F6

E. coli	14.1±0.03	15.5±0.1	16.4±0.03	16.3±0.04	18.5±0.005	19.5±0.04
Staphylococcus aureus	18.5±0.04	19.0±0.05	20.1±0.1	19.5±0.03	20.3±0.02	20.5±0.03
Candida albicans	15.51±0.03	15.7±0.05	16.01±0.03	12.5±0.05	13.7±0.01	16.3±0.02
Aspergillus niger	13.2±0.01	13.0±0.04	13.5±0.06	12.3±0.06	13.3±0.04	14.7±0.03

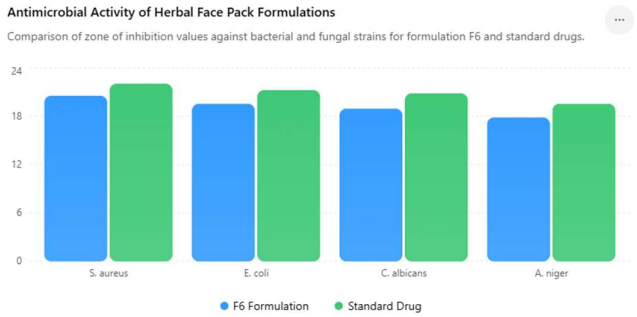


Figure 9: Comparative antimicrobial activity of formulations.

3.8 Antioxidant Activity

The antioxidant activity of the optimized formulation was assessed using the DPPH radical scavenging assay.

A concentration-dependent increase in radical scavenging activity was observed with increasing formulation concentration. The optimized formulation demonstrated substantial antioxidant activity, indicating the ability of the incorporated phytochemicals to neutralize reactive oxygen species implicated in acne-associated inflammation and tissue damage.

The observed antioxidant potential is likely associated with the high content of flavonoids, phenolic acids, and terpenoids extracted from the selected medicinal plants.

Table 8. DPPH radical scavenging activity.

Concentration (µg/mL)	Absorbance	% Inhibition (Mean ± SD)
10	0.692	18.24 ± 0.03
20	0.601	29.45 ± 0.04
30	0.512	38.12 ± 0.03
40	0.463	43.41 ± 0.02
50	0.398	52.84 ± 0.04
Standard (Ascorbic acid)	0.285	71.20 ± 0.03

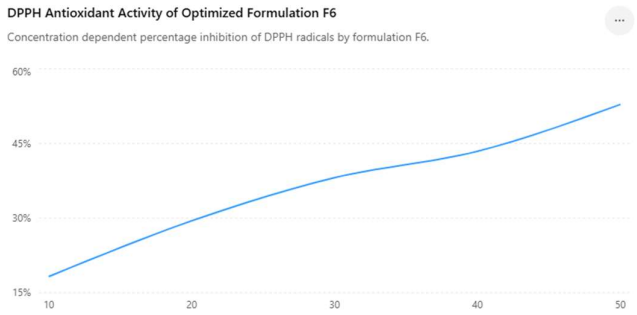


Figure 10. Concentration-dependent antioxidant activity of the optimized formulation.

3.9 Anti-inflammatory Activity

The optimized formulation exhibited significant inhibition of bovine serum albumin denaturation, indicating potent anti-inflammatory activity.

Protein denaturation inhibition increased progressively with increasing formulation concentration and was comparable with the standard anti-inflammatory drug at higher concentrations. The anti-inflammatory effect is likely mediated through inhibition of inflammatory protein denaturation and suppression of oxidative stress by phenolic constituents.

Table 09. Anti-inflammatory activity.

Concentration (µg/mL)	% Inhibition (Mean ± SD)

10	32.45 ± 0.03
20	45.82 ± 0.04
30	58.64 ± 0.03
40	71.28 ± 0.02
50	82.00 ± 0.03
Standard (Diclofenac Sodium)	91.40 ± 0.02

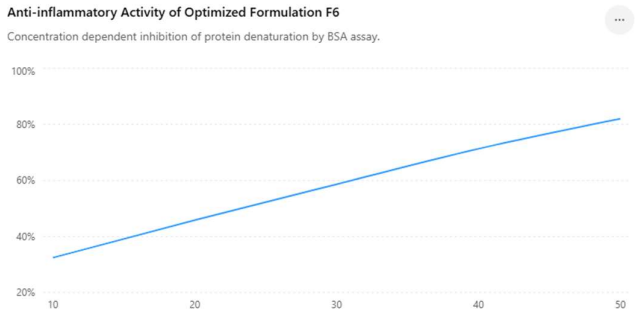


Figure 11. Protein denaturation inhibition by the optimized formulation.

3.10 Cytotoxicity Study

The cytotoxic potential of the optimized formulation was evaluated using the brine shrimp lethality assay. Only minimal mortality of nauplii was observed even at higher concentrations, indicating low cytotoxicity and favorable safety characteristics. The calculated LC_{50} value further confirmed the biocompatibility of the polyherbal formulation. These findings support the suitability of the developed formulation for topical dermatological application.

Table 10. Cytotoxicity study.

Drug	**Conc. of Facepack µg/ml**	**Total no. Shrimps used/tube**	**Shrimp Survived**			**Total No. of Shrimp Survived**	**Percentage mortality**
Control	-	30	10	10	10	30	0.00%
Sample	1	30	10	10	10	30	0.00%
	10	30	10	09	09	28	0.067%
	100	30	09	09	09	27	0.1%

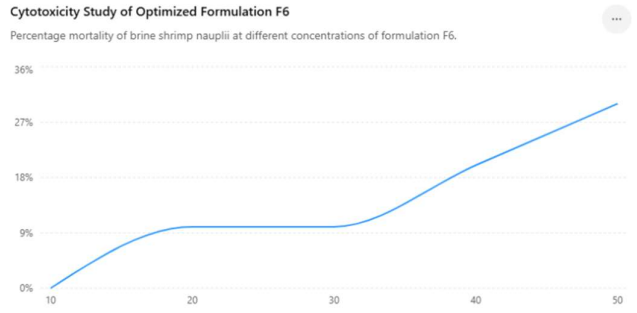


Figure 12. Brine shrimp lethality assay.

3.11 Stability Study

Accelerated stability testing demonstrated that the optimized formulation retained its physicochemical properties throughout the storage period. No significant changes were observed in appearance, color, odor, pH, viscosity, spreadability, or microbial contamination after storage under accelerated conditions. These observations indicate excellent formulation stability and compatibility of the selected ingredients.

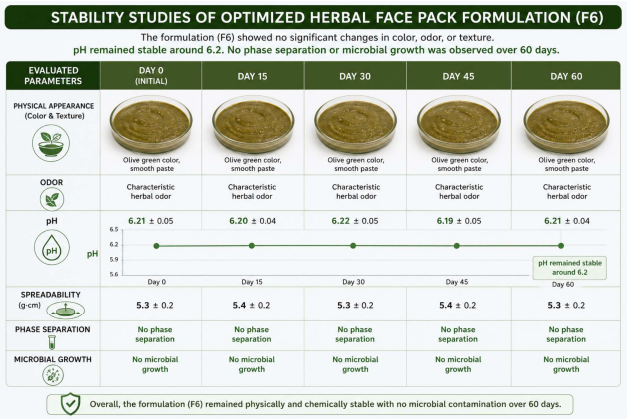


Figure 13: Stability profile of the optimized formulation during storage.

4. Discussion

The present investigation successfully developed and comprehensively evaluated a polyherbal anti-acne face pack incorporating *Azadirachta indica*, *Ocimum sanctum*, *Piper betle*, *Rosmarinus officinalis*, *Cucumis sativus*, and *Aloe vera*. The formulation was designed to exploit the complementary antimicrobial, antioxidant, anti-inflammatory, and skin-protective properties of these medicinal plants to provide a multifunctional topical system for acne management. The experimental findings demonstrated that the optimized formulation (F6) possessed desirable physicochemical characteristics together with significant biological activities, supporting its potential as a safe and effective herbal alternative to conventional anti-acne formulations.

The pharmacognostic evaluation confirmed the identity, purity, and quality of all selected medicinal plants before formulation development. Appropriate moisture content, ash values, and extractive values indicated that the crude drugs complied with standard quality parameters and were suitable for pharmaceutical use. Such quality control measures are essential because variations in moisture content or inorganic contaminants may directly influence phytochemical composition, formulation stability, and biological activity. Similar observations have been reported in pharmacognostic investigations of medicinal plants employed in herbal dermatological formulations, emphasizing the importance of standardization for ensuring batch-to-batch consistency.

Extraction efficiency varied considerably according to solvent polarity. Ethanol and methanol produced substantially higher extractive yields than water, chloroform, and ethyl acetate, indicating their greater ability to dissolve polar phytoconstituents such as flavonoids, phenolic compounds, glycosides, and tannins. These findings agree with numerous reports demonstrating that hydroalcoholic solvents provide superior recovery of antioxidant phytochemicals from medicinal plants. Because phenolic compounds possess multiple hydroxyl groups capable of forming hydrogen bonds with polar solvents, ethanol generally remains one of the preferred extraction media for pharmaceutical and cosmeceutical applications. The higher extraction efficiency observed in the present study therefore contributed to the enhanced biological performance of the optimized formulation.

Preliminary phytochemical screening further supported this observation by demonstrating abundant flavonoids, phenolics, tannins, terpenoids, alkaloids, saponins, and glycosides in the ethanolic and methanolic extracts. These phytochemical classes are well recognized for their synergistic pharmacological activities. Flavonoids and phenolic acids are potent antioxidants that scavenge reactive oxygen species generated during acne-associated inflammation. Terpenoids and essential oils exhibit broad-spectrum antimicrobial activity through disruption of microbial membranes and inhibition of biofilm formation, whereas tannins contribute astringent and antimicrobial properties that help reduce excessive sebum and microbial colonization. Saponins enhance cleansing action and may improve the penetration of active phytoconstituents into superficial skin layers. Consequently, the diverse phytochemical profile observed in the selected medicinal plants provides a strong scientific basis for their combined incorporation into a polyherbal anti-acne formulation.

The physicochemical evaluation demonstrated that all prepared formulations possessed acceptable cosmetic characteristics. The optimized formulation exhibited an appropriate pH within the physiological range of healthy human skin, which is particularly important for maintaining epidermal barrier integrity and preventing irritation. Preservation of the skin's mildly acidic environment also inhibits the proliferation of pathogenic microorganisms and supports the growth of beneficial commensal flora. The viscosity and spreadability values indicated favorable rheological behavior, ensuring easy application, uniform distribution over the skin surface, and prolonged residence time. These characteristics contribute significantly to patient acceptability and therapeutic performance because topical formulations that are excessively viscous

may be difficult to spread, whereas low-viscosity formulations may exhibit poor retention on the skin. The excellent antimicrobial activity demonstrated by the optimized formulation represents one of the principal findings of this investigation. The formulation effectively inhibited both bacterial and fungal microorganisms commonly implicated in dermatological infections. Although *Cutibacterium acnes* is the principal microorganism associated with acne pathogenesis, *Staphylococcus aureus* frequently contributes to secondary skin infections and inflammatory complications. The substantial inhibition observed against *Staphylococcus aureus* therefore suggests that the developed formulation may reduce bacterial colonization while simultaneously minimizing inflammation. The antifungal activity observed against *Candida albicans* and *Aspergillus niger* further broadens the dermatological applicability of the formulation. These antimicrobial effects may be attributed to the combined action of neem limonoids, eugenol from *Ocimum sanctum*, hydroxychavicol from *Piper betle*, rosmarinic acid from rosemary, and phenolic constituents present in cucumber and *Aloe vera*. The simultaneous presence of multiple antimicrobial phytochemicals reduces the likelihood of resistance development compared with single-agent antibiotic therapy.

Oxidative stress is increasingly recognized as an important contributor to acne pathogenesis because reactive oxygen species stimulate lipid peroxidation, activate inflammatory signaling pathways, and promote tissue injury. The optimized formulation exhibited marked concentration-dependent antioxidant activity in the DPPH radical scavenging assay, indicating efficient free radical neutralization. This antioxidant effect is primarily attributable to the abundant phenolic compounds and flavonoids identified during phytochemical screening. These compounds donate hydrogen atoms or electrons to stabilize free radicals, thereby interrupting oxidative chain reactions responsible for inflammatory tissue damage. The observed antioxidant activity therefore complements the antimicrobial action by reducing oxidative stress associated with acne lesions and accelerating tissue repair.

The anti-inflammatory activity demonstrated by inhibition of protein denaturation provides additional evidence supporting the therapeutic potential of the developed formulation. Protein denaturation is an important mechanism involved in inflammatory tissue injury, and inhibition of this process reflects the ability of bioactive compounds to suppress inflammatory responses. The optimized formulation exhibited concentration-dependent inhibition comparable to the standard reference drug, suggesting that the incorporated phytochemicals effectively interfere with inflammatory pathways. Phenolic compounds, flavonoids, terpenoids, and triterpenoids have previously been reported to suppress cyclooxygenase activity, inhibit nuclear factor-kappa B (NF-κB) activation, and reduce the production of pro-inflammatory cytokines including tumor necrosis factor-α, interleukin-1β, and interleukin-6. These mechanisms collectively contribute to reduced erythema, edema, pain, and tissue damage associated with inflammatory acne lesions.

An important advantage of the developed formulation is its favorable safety profile. The absence of significant skin irritation together with negligible cytotoxicity observed during the brine shrimp lethality assay suggests excellent biocompatibility of the selected herbal ingredients. Unlike many conventional anti-acne medications that produce dryness, peeling, burning sensations, or contact dermatitis, the optimized formulation combines therapeutic efficacy with moisturizing and soothing effects provided by *Aloe vera* and cucumber extract. These properties are particularly valuable for improving patient adherence during long-term acne therapy.

The accelerated stability study demonstrated that the optimized formulation maintained its organoleptic characteristics, pH, viscosity, spreadability, and biological integrity throughout the storage period. Stable physicochemical characteristics indicate satisfactory compatibility among the incorporated herbal ingredients and excipients. This stability is essential for pharmaceutical commercialization because instability may reduce therapeutic efficacy and consumer acceptance during storage.

The enhanced biological activity observed in the optimized formulation is likely attributable to synergistic interactions among the incorporated medicinal plants rather than the activity of any single botanical component. Polyherbal formulations frequently demonstrate greater therapeutic efficacy because different phytochemicals simultaneously target multiple pathological mechanisms. In the present formulation, antimicrobial compounds reduce microbial colonization, antioxidants minimize oxidative stress, anti-inflammatory constituents suppress inflammatory mediators, while wound-healing phytochemicals promote epidermal regeneration and restoration of skin barrier function. This multitarget approach is particularly advantageous for managing acne vulgaris, a disorder characterized by complex and interconnected pathological processes.

Despite these promising findings, certain limitations should be acknowledged. The biological evaluations were restricted primarily to in vitro assays, which cannot completely reproduce the complex physiological environment of human skin. Furthermore, quantitative phytochemical analysis and chromatographic standardization of major marker compounds were not performed. Future investigations should include high-performance

liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), or gas chromatography-mass spectrometry (GC-MS) profiling to establish chemical fingerprints of the optimized formulation. Additional studies involving ex vivo skin permeation, in vivo animal models, randomized clinical trials, and long-term dermatological safety evaluations will further strengthen the evidence supporting clinical application.

Overall, the findings of the present study demonstrate that the optimized polyherbal face pack possesses desirable pharmaceutical characteristics together with significant antimicrobial, antioxidant, anti-inflammatory, and safety profiles. The multifunctional mechanism of action, combined with the use of naturally derived medicinal plants, suggests that this formulation represents a promising alternative to conventional anti-acne therapies and may contribute to the development of safer and more sustainable herbal cosmeceutical products.

5. Conclusion

The present study successfully developed and comprehensively evaluated a novel polyherbal anti-acne face pack incorporating *Azadirachta indica*, *Ocimum sanctum*, *Piper betle*, *Rosmarinus officinalis*, *Cucumis sativus*, and *Aloe vera*. The selected medicinal plants were standardized through pharmacognostic evaluation and phytochemical screening, confirming the presence of diverse bioactive constituents with well-established antimicrobial, antioxidant, and anti-inflammatory properties. Among the prepared formulations, F6 exhibited the most favorable physicochemical characteristics, including appropriate pH, desirable viscosity, excellent spreadability, homogeneity, and satisfactory stability, indicating its suitability for topical application.

The optimized formulation demonstrated significant antimicrobial activity against selected bacterial and fungal pathogens, potent free radical scavenging activity in the DPPH assay, marked inhibition of protein denaturation, and negligible cytotoxicity, suggesting an excellent balance between efficacy and safety. These therapeutic effects are likely attributable to the synergistic interaction of multiple phytoconstituents, including flavonoids, phenolics, terpenoids, tannins, alkaloids, and saponins, which collectively target several pathogenic mechanisms involved in acne vulgaris, such as microbial colonization, oxidative stress, inflammation, and impaired skin repair.

Overall, the findings indicate that the developed polyherbal face pack represents a promising natural alternative to conventional topical anti-acne therapies. The formulation combines therapeutic efficacy with cosmetic acceptability and may contribute to the growing demand for safe, sustainable, and plant-based dermatological products. Nevertheless, further quantitative phytochemical characterization, ex vivo skin permeation studies, mechanistic investigations, and randomized clinical trials are recommended to validate its clinical effectiveness and facilitate future commercialization.

6. Study Limitations

Although the present study demonstrated promising pharmaceutical and biological properties, several limitations should be acknowledged.

- The antimicrobial evaluation did not include **Cutibacterium acnes**, the principal microorganism implicated in acne vulgaris.
- The biological activities were assessed using **in vitro** experimental models, which cannot completely replicate the complexity of human skin.
- Quantitative estimation of total phenolic content, total flavonoid content, and marker phytoconstituents was not performed.
- Advanced analytical techniques such as HPLC, LC–MS/MS, GC–MS, FTIR, or SEM characterization were not included.
- Ex vivo skin permeation and skin retention studies were not conducted.
- Clinical evaluation in acne patients was beyond the scope of the present investigation.

These limitations provide opportunities for future research aimed at further validating the therapeutic potential of the developed formulation.

7. Future Perspectives

Future investigations should focus on:

- Standardization of the optimized formulation using HPLC or LC–MS fingerprinting.
- Quantitative estimation of total phenolic and flavonoid contents.
- Mechanistic studies involving inflammatory biomarkers such as TNF-α, IL-1β, IL-6, COX-2, and NF-κB.
- Ex vivo permeation studies using Franz diffusion cells.
- In vivo evaluation using validated acne animal models.
- Randomized controlled clinical trials to establish safety and therapeutic efficacy in human volunteers.
- Development of advanced topical delivery systems such as nanoemulgels, nanostructured lipid carriers, liposomes, or hydrogel-based facial masks to improve skin penetration and patient compliance.
- Long-term stability studies under ICH storage conditions and scale-up for industrial production.

CRediT Authorship Contribution Statement
First Author: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing—original draft. **Co-authors:** Resources, Investigation, Data interpretation, Visualization, Writing—review and editing.

Funding
The authors declare that no external financial support was received for conducting this research.

Institutional Review Board Statement
The study was conducted in accordance with institutional guidelines. Where applicable, experimental procedures involving biological materials were approved by the Institutional Ethics Committee.

Informed Consent Statement
Not applicable.

Data Availability Statement
The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest
The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Acknowledgements
The authors sincerely acknowledge the College of Pharmacy or providing the necessary laboratory facilities and technical support to carry out this research. The authors are also grateful to all faculty members and laboratory staff for their valuable assistance during the experimental work.

References

1. Zaenglein AL. Acne vulgaris. N Engl J Med. 2018;379(14):1343–1352.
2. Bhate K, Williams HC. Epidemiology of acne vulgaris. Br J Dermatol. 2013;168(3):474–485.
3. Tan JKL, Bhate K. A global perspective on the epidemiology of acne. Br J Dermatol. 2015;172(Suppl 1):3–12.
4. Proença AC, Luís Â, Duarte AP. The role of herbal medicine in the treatment of acne vulgaris: A systematic review of clinical trials. Evid Based Complement Alternat Med. 2022;2022:2011945.
5. Ramsis T, Selim HMRM, Elseedy H, Fayed EA. The role of current synthetic and possible plant and marine phytochemical compounds in the treatment of acne. RSC Adv. 2024;14:24287–24321.
6. Gaur PK, Mishra R, Kaushik R, Verma KK, Kumar N, Lata K. Polyherbal antiacne gel: In vitro antibacterial activity and efficacy evaluation against *Cutibacterium acnes*. Assay Drug Dev Technol. 2024.
7. Aggarwal M, Vatsa E, Chaudhary N, Chandel S, Gautam S. Formulation and evaluation of polyherbal face pack. Res J Pharm Technol. 2024;17(6):2481–2485.
8. Khan N, Pawar AP, Patil S, Patil PR, Patil PV, Patil PN, et al. Formulation and evaluation of polyherbal anti-acne patch. MGM J Med Sci. 2024;11(2):234–241.
9. Sharma B, Sharma PK, Kannojia P, Kumar S, Verma SK. Development and assessment of Ayurvedic herbal face pack "Mukha Lepa" for skin enhancement. Curr Cosmet Sci. 2025;4:1–6.
10. Popaniya HS, Vaja PN, Tank CJ, Suva H, Rathod D. A review on herbal components used for polyherbal facewash. Asian J Pharm Res. 2024;14(3):319–325.
11. Kumar R, Komal. Formulation and evaluation of herbal face pack. Asian J Pharm Res. 2021;11(1):9–12.
12. Shimpi AA, Pawara AS. A review on herbal face pack. Res J Pharmacol Pharmacodyn. 2022;14(3):146–150.
13. Kapoor VP. Herbal cosmetics for skin and hair care. Indian J Nat Prod Resour. 2005;4:306–314.
14. Saxena R, Pal Y, Pranaywal. In-house preparation and standardization of herbal face pack. Open Dermatol J. 2017;11:72–80.
15. Yadav N, Yadav R. Preparation and evaluation of herbal face pack. Int J Recent Sci Res. 2015;6(6):4334–4337.
16. Saraf S, Saraf S. Cosmetics: A Practical Manual. 2nd ed. New Delhi: PharmaMed Press; 2005.
17. Bhosale KP, Ugale PD, Daingade PS. Formulation and evaluation of herbal face pack. J Drug Deliv Ther. 2025;15:84–92.
18. Liu G, Li J, Wang L, Zhang L, Yang T, Guo Y, et al. Fast-dissolving nanofibrous facial masks for delivering skincare ingredients. Colloids Surf B Biointerfaces. 2025;245:114770.
19. Dalavi PR, Khan ZK. Formulation and evaluation of herbal face pack. Int J Sci Res Technol. 2025;2(4):236–243.
20. Madihalli S, Masti SP, Eelager MP, Chougale RB, Anilkumar BM, Priyadarshini AN. Methylcellulose/chitosan bioactive films enriched with *Achyranthes aspera* leaf extract for cosmetic face mask applications. Int J Biol Macromol. 2025;303:140611.

21. Saeed HAM, Yang H. The versatile world of cosmetic facial masks in skincare: Fabrication, properties and future directions. J Drug Deliv Sci Technol. 2025;108:106895.
22. Nanaware J, Pawar SS, Abhang P. Evaluation of physicochemical and anti-inflammatory properties of commercially available herbal face packs. Bull Pure Appl Sci Zool. 2024;43B:598–606.
23. Jain A, Gautam SP, Gupta Y, Jain S. Development and characterization of ketoconazole emulgel for topical drug delivery. Der Pharm Sin. 2010;1(3):221–231.
24. Singla V, Saini S, Joshi B, Rana AC. Emulgel: A new platform for topical drug delivery. Int J Pharm Bio Sci. 2012;3(1):485–498.
25. Kuller R, Saini S, Rana AC. Emulgel: A surrogate approach for topical use of hydrophobic drugs. Int J Pharm Bio Sci. 2011;1(3):117–128.
26. Mishra AN. Controlled and Novel Drug Delivery. 4th ed. New Delhi: CBS Publishers; 2003.
27. Swarbrick J. Encyclopedia of Pharmaceutical Technology. 3rd ed. New York: Informa Healthcare; 2007.
28. Ranade VV, Hollinger MA. Drug Delivery Systems. 2nd ed. Boca Raton: CRC Press; 2010.
29. Tortora GJ, Derrickson B. Principles of Anatomy and Physiology. 11th ed. Hoboken: John Wiley & Sons; 2007.
30. Debjit B, Harish G, Pragati K, Duraivel S, Sampath KP. Recent advances in novel topical drug delivery system. Pharm Innov. 2012;1:9–16.
31. Devada P, Jain A, Vyas N, Jain S. Development of antifungal emulsion-based gel for topical fungal infections. Int J Pharm Res Dev. 2011;3(2):18–25.
32. Kaila YN, Guy RH. Modeling transdermal drug release. Adv Drug Deliv Rev. 2001;48:159–172.
33. Ayub CA, Gomes AD, Lima MV, Vianna CD, Ferreira LM. Topical delivery of fluconazole: In vitro skin penetration and permeation using emulsions as dosage forms. Drug Dev Ind Pharm. 2007;33:273–280.
34. Singh PB, Choudhary PK. Penetration enhancers for transdermal drug delivery of systemic agents. J Pharm Res. 2007;6:44–50.
35. Kumar D, Rathi L, Tripathi A, Maddheshiya YP. A review on oral mucosal drug delivery system. Int J Pharm Sci Res. 2010;1(5):50–56.

Abbreviations

Abbreviation	Full Form
DPPH	2,2-Diphenyl-1-picrylhydrazyl
BSA	Bovine Serum Albumin
AR	Analytical Reagent
SD	Standard Deviation
ANOVA	Analysis of Variance
HPLC	High-Performance Liquid Chromatography
LC–MS	Liquid Chromatography–Mass Spectrometry
SEM	Scanning Electron Microscopy
FTIR	Fourier Transform Infrared Spectroscopy
ROS	Reactive Oxygen Species
NF-κB	Nuclear Factor-kappa B
TNF-α	Tumor Necrosis Factor-alpha
IL	Interleukin