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FORMULATION AND EVALUATION OF POLYHERBAL GEL FOR MANAGEMENT OF ACNE AND HYPERPIGMENTATION

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ABSTRACT

Acne vulgaris is one of the most common chronic inflammatory skin disorders affecting adolescents and adults worldwide. It is characterized by pimples, redness, swelling, hyperpigmentation, and irritation caused mainly due to excessive sebum production, follicular blockage, bacterial growth, and inflammation. The major bacterium involved in acne formation is Cutibacterium acnes, formerly known as Propionibacterium acnes. When excess sebum, dead skin cells, and blocked pores accumulate on the skin surface, this bacterium proliferates rapidly and breaks down sebum into free fatty acids, leading to inflammation, redness, swelling, and acne lesions. Several enzymes also play an important role in acne pathogenesis, including lipase, protease, hyaluronidase, matrix metalloproteinases (MMPs), and 5-alpha reductase. Lipase produced by C. acnes hydrolyzes triglycerides into irritating fatty acids, while protease and hyaluronidase contribute to tissue damage and spread of inflammation. Matrix metalloproteinases are associated with collagen degradation and acne scar formation, whereas 5-alpha reductase increases sebum secretion by converting testosterone into dihydrotestosterone (DHT). The current research is based on the development and evaluation of a polyherbal gel formulation for acne and hyperpigmentation treatment, where herbs such as neem, turmeric, Glycyrrhiza glabra, and Aloe vera have been used. Herbs contain various properties such as antibacterial, anti-inflammatory, antioxidant, depigmenting, and wound healing abilities that could aid in the prevention of acne bacteria and improving skin conditions with least adverse effects. The formulation of polyherbal gel has been achieved by utilizing the dispersion technique, where Carbopol 934 has been used as a gelling agent. Physiochemical evaluation was performed to check the appearance, pH value, homogeneity, spreadability, viscosity, washability, extrudability, stability, and antimicrobial activity of the gel. The formulated polyherbal gel exhibited good physical characteristics and possessed anti-acne activity against C. acnes. Therefore, the developed formulation may serve as a safe, effective, and economical herbal alternative for the treatment and management of acne vulgaris and associated hyperpigmentation.[1,9]

KEYWORDS: Cutibacterium Acne, Neem Extract, Hyaluronidase, Matrix Metalloproteinases, 5-Alpha Reductase, Polyherbal gel, Hyperpigmentation, Carbopol 934.

INTRODUCTION

Acne vulgaris is a multifactorial inflammatory skin disorder that primarily affects the pilosebaceous follicles. It is commonly observed during adolescence due to hormonal changes, although adults may also experience persistent or recurrent acne lesions. The condition predominantly appears on sebaceous gland–rich areas such as the face, upper chest, shoulders, and back. Clinically, acne may present as comedones, papules, pustules, nodules, cysts, and, in severe cases, permanent scarring or post-inflammatory hyperpigmentation. Beyond its physical manifestations, acne can negatively

influence emotional well-being, self-esteem, and social confidence, making it a significant dermatological and psychological concern. Traditional systems of medicine have utilized herbal remedies for the treatment of skin disorders for centuries. Ancient Egyptian, Greek, Chinese, and Ayurvedic medical practices documented skin eruptions comparable to acne and recommended plant-derived preparations for improving skin health. In Ayurvedic literature, acne-like lesions were described as "**Yuvanpidika**," a condition commonly associated with puberty. Herbs such as turmeric, neem, aloe vera, tulsi, sandalwood, and liquorice were frequently employed because of their soothing, antimicrobial, and complexion-enhancing properties. Many of these medicinal plants continue to attract scientific attention due to their therapeutic potential and comparatively lower incidence of adverse effects than synthetic agents.[8]

The development of acne involves several interconnected pathogenic mechanisms. Excessive sebum secretion, abnormal keratinization of hair follicles, microbial colonization, inflammatory responses, oxidative stress, and hormonal influences collectively contribute to lesion formation. Elevated androgen levels during puberty stimulate sebaceous gland activity, resulting in increased oil production. Simultaneously, accumulation of keratinized cells within follicles obstructs pores and promotes comedone formation. The blocked follicular environment Favor's the proliferation of *Cutibacterium acnes*, a bacterium strongly associated with acne inflammation. *C. acnes* produce enzymes and inflammatory mediators that aggravate tissue damage and immune responses. Lipase enzymes convert sebum triglycerides into irritating free fatty acids, thereby promoting inflammation of follicular walls. Additional enzymes such as proteases and hyaluronidases contribute to tissue breakdown and facilitate the spread of inflammatory reactions into deeper skin layers. Matrix metalloproteinases activated during inflammation are also implicated in collagen degradation and scar formation. Another important factor involved in acne progression is the enzyme 5-alpha reductase, which converts testosterone into dihydrotestosterone, a potent androgen that further enhances sebaceous gland activity. Acne lesions may be classified as non-inflammatory or inflammatory depending on the severity of follicular obstruction and associated inflammation. Non-inflammatory lesions include open and closed comedones. Closed comedones, commonly called whiteheads, develop when clogged follicles remain covered by skin, whereas open comedones or blackheads form when accumulated material is exposed to air and undergoes oxidation. Inflammatory lesions include papules, pustules, nodules, and cysts. Papules are erythematous raised lesions, while pustules contain purulent material resulting from inflammatory and bacterial processes.

Nodules and cysts are deeper lesions that are often painful and may heal with scarring. Several factors including genetics, hormonal imbalance, stress, dietary habits, cosmetic products, and environmental conditions may influence disease severity. Post-inflammatory hyperpigmentation is a common consequence of acne, particularly in individuals with darker skin tones. It occurs when inflammatory mediators stimulate melanocytes to produce excessive melanin during the healing process. These pigmented lesions may persist for prolonged periods and appear as brown, red, or dark discolorations on the skin.[10]

The enzyme tyrosinase plays a central role in melanin biosynthesis; therefore, inhibition of tyrosinase activity represents an important therapeutic strategy for reducing hyperpigmentation and improving skin appearance. Conventional management of acne includes topical and systemic therapies such as antibiotics, retinoids, benzoyl peroxide, hormonal agents, salicylic acid, and corticosteroids. Antibiotics including clindamycin, erythromycin, doxycycline, and tetracycline are commonly prescribed to suppress bacterial growth and reduce inflammation. Despite their effectiveness, prolonged use of these agents may lead to several limitations, including antibiotic resistance, recurrence after discontinuation, skin irritation, dryness, peeling, photosensitivity, gastrointestinal disturbances, and disruption of normal skin microbiota. These concerns have encouraged researchers to investigate safer and more sustainable alternatives derived from natural sources.

Herbal medicines have emerged as promising candidates in dermatological research because of their broad spectrum of biological activities. Medicinal plants contain numerous phytochemicals such as flavonoids, alkaloids, phenolic compounds, tannins, terpenoids, and curcuminoids that exhibit antimicrobial, antioxidant, anti-inflammatory, and wound-healing effects. Polyherbal formulations are particularly advantageous because they combine multiple plant extracts that may act synergistically to target different stages of acne pathogenesis while minimizing adverse reactions. Among various topical dosage forms, gels are widely preferred for acne management due to their non-greasy nature, ease of application, transparency, and patient acceptability.

Gel formulations spread easily over the skin, provide a cooling sensation, and facilitate direct delivery of active constituents to affected areas. They are especially suitable for oily and acne-prone skin because they do not excessively occlude pores. Carbopol-based gels are frequently used in pharmaceutical formulations owing to their desirable viscosity, stability, clarity, and compatibility with herbal extracts. The present work focuses on the development and evaluation of a polyherbal topical gel containing turmeric, neem, aloe vera, and liquorice for the management of acne and

associated hyperpigmentation. Neem is recognized for its antimicrobial and anti-inflammatory properties against acne-associated microorganisms. Aloe vera contributes moisturizing, soothing, and wound-healing effects that may reduce irritation and redness. Liquorice is valued for its skin-brightening and anti-inflammatory activities, making it useful in the management of post-inflammatory pigmentation.[16,11]

Turmeric (Curcuma longa Linn.) is considered one of the most significant medicinal plants in dermatological therapy. Its principal bioactive constituent, curcumin, demonstrates potent antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties. Curcumin may reduce oxidative stress associated with acne lesions and suppress inflammatory mediators involved in erythema and swelling. Additionally, it has been reported to inhibit tyrosinase activity, thereby reducing melanin synthesis and improving post-acne hyperpigmentation. The compound also supports tissue repair and protects skin cells from oxidative damage caused by free radicals.

Natural depigmenting agents can generally be categorized into antioxidants, tyrosinase inhibitors, and multifunctional agents. Antioxidants help neutralize reactive oxygen species and minimize oxidative tissue injury, whereas tyrosinase inhibitors directly reduce melanin formation by interfering with melanogenic pathways. Multifunctional agents exhibit combined antioxidant, anti-inflammatory, antimicrobial, and skin-repairing properties. Curcumin is considered a multifunctional phytochemical because it acts through several mechanisms relevant to both acne control and hyperpigmentation reduction.

Therefore, the formulation of a polyherbal anti-acne gel using medicinal plant extracts may provide an effective, economical, and safer alternative to conventional therapies. By combining antimicrobial, anti-inflammatory, wound-healing, depigmenting activities, herbal formulations have the potential to improve acne symptoms and associated pigmentation while reducing the likelihood of adverse effects commonly associated with synthetic drugs. **In Figure No: 1, Table No. 1**[8]

Table No. 1: Causes of Acne and Hyperpigmentation.

Causes of Acne Vulgaris	Causes of Hyperpigmentation
Excess sebum (oil) production	Excess melanin production
Follicular hyperkeratinization	Post-inflammatory pigmentation after acne or skin injury
Genetic predisposition	Skin inflammation and irritation
Growth of Cutibacterium acnes bacteria	Ultraviolet (UV) radiation exposure
Hormonal imbalance	Hormonal changes or imbalance
Inflammation of sebaceous glands	Increased tyrosinase enzyme activity
Poor skin hygiene	Allergic skin reactions

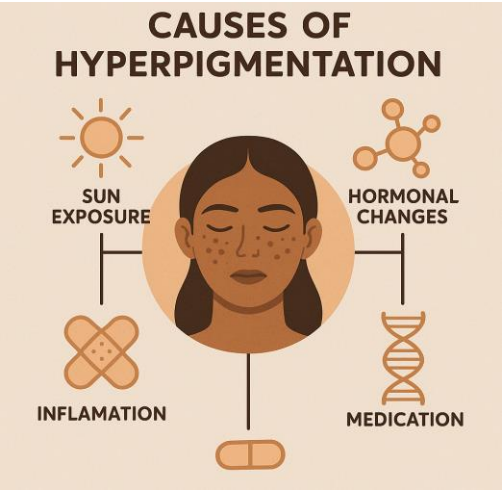


Figure No. 1: Causes of Hyperpigmentation.

Literature Review

- Previous studies have reported the development of various herbal and polyherbal formulations for the treatment of acne and skin pigmentation disorders. **Chandrasekar** and **Kumar** formulated a polyherbal anti-acne gel containing Aloe vera and turmeric and evaluated its physical properties and **anti-acne activity**. The study demonstrated good anti-inflammatory and anti-acne potential; however, it mainly focused on acne treatment and did not deeply investigate hyperpigmentation or tyrosinase inhibition. The present study improves this limitation by developing a polyherbal gel aimed at managing both acne vulgaris and post-acne hyperpigmentation simultaneously.
- **Chellathurai et al**. developed a polyherbal topical gel using **Aloe barbadensis and Vigna radiata for acne** treatment. Their formulation showed

satisfactory antimicrobial activity and acceptable physicochemical characteristics. However, the study did not include herbs specifically targeting pigmentation reduction such as **turmeric or licorice**. In the current work, turmeric and licorice are incorporated to provide additional antioxidant and tyrosinase inhibitory activity for reducing dark spots associated with acne.

- **Mhaske et al**. prepared a dual-gelled **polyherbal anti-acne formulation** and reported it to be stable, safe, and effective against acne lesions. Although the formulation was beneficial in reducing acne symptoms, it primarily targeted acne and not **post-inflammatory pigmentation.** The present study addresses this limitation by focusing on both inflammatory acne and acne-induced hyperpigmentation through a multi-herbal approach.
- **Shrotriya et al**. prepared **a curcumin-based topical** gel system intended mainly for pigmentation disorders and studied its tyrosinase inhibitory activity and skin deposition characteristics. The study highlighted the role of **curcumin in reducing melanin formation** but did not combine multiple anti-acne herbs within the same formulation. The current study improves this by combining turmeric with **neem, Aloe vera, and licorice** to achieve antibacterial, anti-inflammatory, antioxidant, and depigmenting effects together in one gel system.
- **Rocchitta et al**. investigated curcumin-inspired derivatives as tyrosinase inhibitors for **controlling melanin synthesis**. Their research mainly focused on enzymatic inhibition and pigmentation mechanisms rather than developing a complete topical herbal formulation. The present study translates the tyrosinase inhibitory concept into a practical polyherbal topical gel intended for routine **acne and pigmentation management.**
- **Parasuraman et al.** explained the concept of **polyherbal formulations in Ayurveda** and discussed how combining multiple medicinal plants may produce synergistic therapeutic effects. However, their work was mainly conceptual and did not involve the preparation of a **specific anti-acne gel formulation**. The present study applies the polyherbal concept practically by developing and evaluating a topical gel specifically designed for acne vulgaris and hyperpigmentation.
- Neem-based herbal therapies have been widely studied because neem possesses antibacterial, anti-inflammatory, and oil-regulating properties effective against acne-causing microorganisms. However, neem alone may not adequately address hyperpigmentation and skin repair. The current study combines neem with turmeric, Aloe vera, and licorice to broaden therapeutic activity and provide improved skin healing and pigmentation reduction.
- **Aloe vera-based formulations** have also been investigated extensively for their soothing, **moisturizing, anti-inflammatory, and wound-healing effects in acne-prone skin.** Despite these benefits, Aloe vera alone has limited direct action on melanin reduction and **tyrosinase inhibition**. The present formulation overcomes this limitation by adding turmeric and licorice, which may help reduce post-acne dark spots and uneven skin tone.
- Researchers studying herbal treatment approaches for hyperpigmentation have reported that plant-derived tyrosinase inhibitors may effectively reduce melanin synthesis and improve skin complexion. However, most studies focused only on pigmentation disorders and did not consider acne pathogenesis, bacterial growth, and inflammation. The present study improves this by designing a gel that simultaneously targets excess sebum, microbial growth, inflammation, and pigmentation.

Novelty

This study presents a novel polyherbal gel formulated for the combined treatment of acne vulgaris and post-inflammatory hyperpigmentation. In contrast to many existing herbal formulations that primarily focus only on acne control, this gel is designed to deliver multiple therapeutic effects through a single topical preparation. The formulation aims to provide antibacterial, anti-inflammatory, antioxidant, wound-healing, and skin-brightening activities simultaneously.

The uniqueness of the formulation lies in the incorporation of medicinal plants such as turmeric, neem, Aloe vera, and liquorice, each contributing distinct pharmacological benefits. Neem is well known for its antibacterial action against acne-causing bacteria, especially *Cutibacterium acnes*. Aloe vera helps soothe the skin, maintain moisture, and support healing of damaged tissue. Turmeric contains curcumin, which possesses antioxidant and anti-inflammatory properties and also helps reduce hyperpigmentation by inhibiting tyrosinase activity. Licorice extract further enhances the formulation through its skin-lightening and anti-inflammatory effects, thereby helping to minimize dark spots and improve overall skin appearance.[6]

Another important feature of this work is the development of a formulation capable of acting through multiple mechanisms involved in acne development. The gel is intended to suppress microbial growth, reduce inflammation and oxidative stress, calm irritated skin, and control excessive melanin production, all of which contribute to acne lesions and pigmentation problems.
The study also emphasizes improved physical stability of the formulation. A Carbopol-based gel system was selected to achieve suitable viscosity, uniform consistency, good homogeneity, and enhanced stability during storage. In addition, the gel has been formulated to remain non-greasy, cosmetically acceptable, and convenient for application on sensitive and acne-prone skin.

The combination of several herbal extracts may also produce a synergistic antimicrobial effect, offering

broader protection than formulations containing a single herb. Along with antibacterial activity, the herbal ingredients contribute antioxidant and anti-inflammatory actions that may improve the overall effectiveness of acne treatment.

Furthermore, the gel is expected to provide good spreadability and compatibility with the skin. Its non-sticky texture and easy washability can improve user convenience and treatment adherence. Natural ingredients such as Aloe vera may also help minimize irritation, dryness, and redness that are commonly associated with synthetic anti-acne products. Overall, the developed polyherbal gel represents a stable, safe, multifunctional, and user-friendly herbal formulation for managing acne vulgaris and related hyperpigmentation.[5]

MATERIALS AND METHODS

DETAIL PROFILE OF MATERIAL USED

1. Neem (Azadirachta Indica)

Synonyms: Nira, Nimb, Vespa, Limba, Nimba

Biological source: Neem consists of the fresh or dried leaves and seed oil of Azadirachta indica belonging to family **Meliaceous**.

Azadirachta indica, widely known as neem or Indian lilac, belongs to the Meliaceae (mahogany) family. The plant originates from the Indian subcontinent and several regions of Southeast Asia. Due to its medicinal and agricultural importance, it is now cultivated in many tropical and subtropical countries worldwide. Neem fruits and seeds are mainly used for extracting neem oil, which has various pharmaceutical and therapeutic applications. The word “nim” comes from the Sanskrit term “nimba” and is commonly used in Hindustani languages. **Figure no 2, 3.**



Figure No. 2: Neem (Azadirachta Indica).

Chemical Constituents: Nimbin, 6-desacetylnimbinene, Nimbiene, Nimbandiol, nimbolide, Quercetin, Ascorbic acid, n-hexaacosanol, amino acid, Nimbin & Nimbidinin.

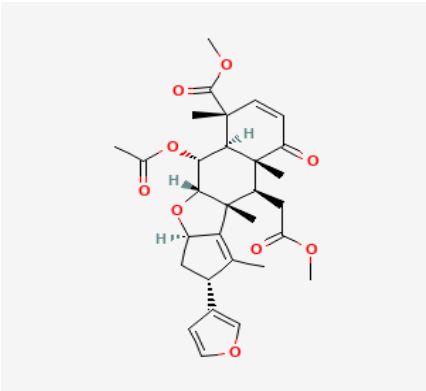


Figure No. 3: Structure of Nimbin.

Geographical source: It is found in India, Pakistan, Afghanistan, Uzbekistan, Dubai, South Africa, and Australia.

Uses

- Skin Care
- Hair Care
- Dental Care
- Antifungal uses
- Immune booster
- Blood purification
- Antidiabetics

2. Turmeric (Curcuma longa Linn.)

Synonyms: Indian saffron, Haldi, Haridra, Turmeric rhizome, Curcuma domestica, yellow ginger.

Biological source: Turmeric consists of the dried rhizomes of Curcuma longa Linn. Which belonging to Family Zingiberaceae.



Figure No. 4: Turmeric (Curcuma longa Linn).

Chemical Constituents: Curcuminoids, Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin, Volatile Oils, Proteins, Carbohydrates, Minerals and Vitamins.

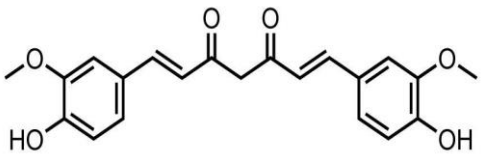


Figure No. 5: Structure of Curcuma longa Linn.

Geographical source: It is found in India, Bangladesh, Korea, Pakistan, Thailand, Indonesia, Sri Lanka, and UAE.

Uses

- Anti-inflammatory
- Antioxidant
- Antimicrobial
- Wound healing
- Tyrosinase inhibitory activity
- Skin brightening and anti-acne formulations.

3. Aloe vera (Aloe barbadensis Miller))

Synonyms: Aloe barbadensis Miller, Aloe vera Linn., Indian Aloe, True Aloe, Medicinal Aloe**.**

Biological source: Aloe vera consists of the dried juice or fresh mucilaginous gel obtained from the leaves of Aloe barbadensis Miller.

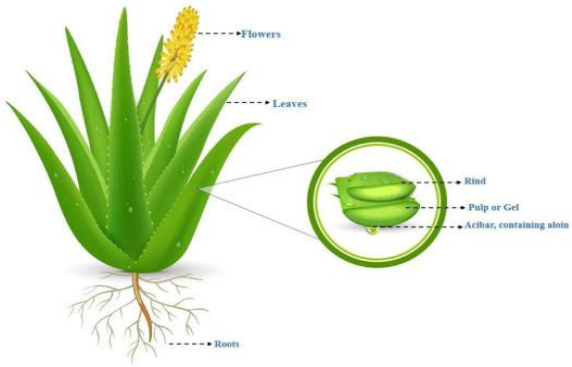


Figure No. 6: Aloe vera (Aloe barbadensis Miller).

Chemical Constituents: Anthraquinones, Aloin, Aloe-emodin, Barbaloin, Polysaccharides, Vitamins, Enzymes, and Amino acid.

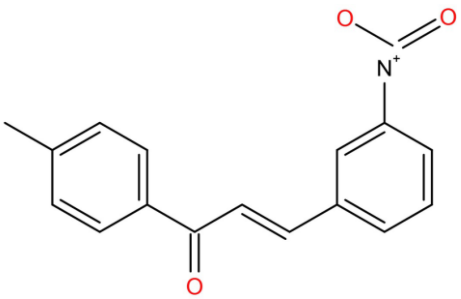


Figure No 7: Structure of Aloe barbadensis Miller.

Geographical source: It is found in India, Indonesia, Pakistan, United states of America, Uzbekistan, Egypt, and Sudan.

Uses

- Moisturizing agent
- Wound healing
- Anti-inflammatory activity
- Skin soothing effect
- Antimicrobial activity

4. Liquorice (Glycyrrhiza glabra Linn.)

Synonyms: Licorice, Sweet wood, Mulethi, Yashtimadhu, Jyeshthamadh.

Biological source: Licorice consists of the dried roots and stolon of Glycyrrhiza glabra Linn. Which belonging to family Fabaceae.



Figure No. 8: Liquorice (Glycyrrhiza glabra Linn).

Chemical Constituents: Triterpenoid Saponins, Glycyrrhizin, Glycyrrhetinic acid, Flavonoids, Liquiritin, Isoliquiritin, Liquiritigenin, and Glabridin.

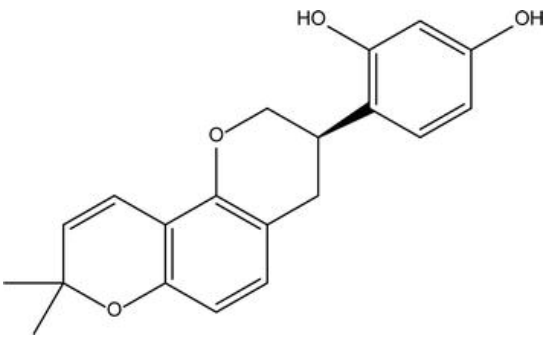


Figure No. 9: Structure of Glycyrrhiza glabra Linn.

Geographical source: It is found in places like India, Iran, Pakistan, Russia, UAE, United states of America, and Uzbekistan.

Uses

- Anti-inflammatory activity
- Skin brightening
- Tyrosinase inhibition
- Antioxidant activity
- Antimicrobial effect
- Soothing agent

METHODS

Procurement of plant material

Standardized herbal extracts of Neem (Azadirachta indica), Turmeric (Curcuma longa), Licorice (Glycyrrhiza glabra), and Aloe vera (Aloe barbadensis Miller) were procured from certified herbal suppliers along with their respective Certificates of Analysis (COA). The supplied extracts complied with the required physicochemical, microbiological, and heavy metal specifications as provided by the manufacturer. The

analytical reports confirmed the identity, purity, quality, and safety of the herbal materials used in the study. The extracts were stored in airtight containers at room temperature in a dry and protected environment until further use.

Since commercially available standardized extracts were utilized in the present investigation, no additional extraction, purification, or isolation procedures were carried out in the laboratory. The herbal extracts were used directly for formulation of the polyherbal gel. Accurately weighed quantities of each extract were separately dispersed in suitable solvent systems such as purified water and propylene glycol to obtain uniform dispersion and proper incorporation into the gel base. Continuous stirring was maintained to avoid lump formation and to ensure homogeneity of the formulation prior to addition into the Carbopol gel matrix.[10]

Evaluation of Extract

Characteristics of Extract: The ethanolic extract of Turmeric, Neem, Liquorice, and Aloe vera As the Extract evaluation was based on the **supplier's COA**, including: Description/colour/odour, Identification, Loss on drying, Ash values, Extractive values, Curcuminoid content, Microbial limits, Heavy metals. **Table no 2**

Table No. 2: Characteristics of Extract.

Parameters	Neem	Turmeric	Aloe vera	Liquorice
Colour	Brown fine	Yellow to orange	Transparent Green Gel	Brown fine
Odour	Characteristic	Characteristic	Characteristic	Characteristic
Identification	Positive	Positive	Positive	Positive
Loss on Drying (LOD)	7.3%	6.5%	6.7%	7.1%
Ash Value	3.1%	2.8%	3.0%	3.4%
Extractive Value	10.5%	10.8%	9.6%	9.7%
Microbial Limits	Absent	Absent	Absent	Absent
Heavy Metals	Within Limits	Within Limits	Within Limits	Within Limits

Phytochemical Screening of Extract: Phytochemical screening of extracts obtained from Azadirachta indica (neem), Curcuma longa (turmeric), Aloe vera, and Glycyrrhiza glabra (liquorice) showed the presence of various important bioactive constituents. Neem extract was found to contain alkaloids, flavonoids, tannins, glycosides, saponins, steroids, and terpenoids which are responsible for its antimicrobial and anti-inflammatory properties. Turmeric extract showed the presence of curcuminoids, flavonoids, tannins, phenolic compounds, and essential oils contributing to its antioxidant and therapeutic activities. Aloe vera extract revealed the presence of anthraquinones, saponins, glycosides, carbohydrates, and amino acids which provide wound healing and soothing effects**. After** this the Turmeric, Neem, Aloe vera, and Liquorice were subjected to preliminary phytochemical testing for the detection of major phytoconstituent such as phenols, tannins, alkaloids, glycoside, flavonoids, etc. **Table 3**[14]

Table No. 3: Phytochemical Screening of Extract.

	Neem	Turmeric	Aloe vera	Liquorice
Phenol	+	+	+	+
Tannins	+	+	+	+
Steroids	+	-	+	+
Alkaloids	+	+	+	+
Glycosides	+	+	+	+
Flavonoids	+	+	+	+

Selection and Optimization of Gelling Agents

Carbopol 934 was employed as the gel-forming polymer in the development of the polyherbal formulation. To determine the most suitable polymer concentration, three experimental batches were prepared using Carbopol 934 at concentrations of 0.5%, 1%, and 1.5% w/w. Propylene glycol served as a permeation enhancer, while methylparaben was included to prevent microbial growth. Triethanolamine was utilized to neutralize the formulation and maintain the desired pH, and purified water was added in sufficient quantity to achieve the final volume. The prepared gels were assessed for physicochemical characteristics such as visual appearance, uniformity, pH, viscosity, spreadability, extrudability, and consistency. Among all formulations, the gel containing 1% Carbopol 934 demonstrated desirable viscosity, satisfactory spreadability, a smooth consistency, appropriate pH, and improved ease of application. Based on these observations, the formulation containing 1% Carbopol 934 was chosen as the optimized gel base for subsequent preparation of the polyherbal gel. **Table no 4, In Figure no 10.**[15]

Table No. 4: Selection and Optimization of Gelling Agents.

Ingredients	F1	F2	F3
Carbopol 934	0.5%	1%	1.5%
Propylene Glycol	5ml	5ml	5ml
Methyl paraben	0.2g	0.2g	0.2g
Triethanolamine	5ml	5ml	5ml
Water	Q. s	Q. s	Q. s



Figure No. 10: Selection of Gelling Agents.

Factorial Design of Formulation

The present study aimed to develop and optimize a polyherbal topical gel for the treatment of acne vulgaris and post-inflammatory hyperpigmentation by incorporating herbal ingredients known for their multiple therapeutic benefits. A systematic experimental design was adopted to obtain a formulation with desirable physicochemical properties and enhanced therapeutic performance. Factorial design is widely used in pharmaceutical research because it allows simultaneous evaluation of different formulation variables and helps identify the most suitable combination of ingredients with fewer experimental runs.

For optimization of the formulation, a (3^2) factorial design was employed. Two independent variables were selected: the concentration of liquorice extract (Factor A) and neem extract (Factor B). These extracts were chosen because of their significant roles in managing acne and pigmentation disorders. Each variable was studied at three levels: low (-1), medium (0), and high (+1). Based on various combinations of these concentration levels, nine different gel formulations (F1–F9) were prepared and evaluated.

Liquorice extract was included due to its skin-lightening, antioxidant, anti-inflammatory, and melanin-inhibiting properties. Active compounds such as glabridin and liquiritin are known to reduce melanin production and improve uneven skin tone associated with acne marks and hyperpigmentation. Different concentrations of liquorice extract were tested to determine the level that could provide effective depigmenting activity while maintaining the stability and consistency of the gel.

Neem extract was selected because of its strong antimicrobial and anti-inflammatory effects. It has been reported to inhibit the growth of acne-associated microorganisms, including *Cutibacterium acnes*, and helps reduce swelling, redness, irritation, and microbial contamination in acne lesions. By varying the concentration of neem extract in the formulations, an attempt was made to achieve optimal antimicrobial effectiveness along with acceptable formulation characteristics.

In addition to therapeutic efficacy, the influence of herbal extract concentrations on the physical properties of the gel was also investigated. All prepared formulations were evaluated for appearance, homogeneity, pH, viscosity, spreadability, extrudability, washability, and stability. The final optimized formulation was selected on the basis of appropriate texture, ease of application, acceptable viscosity, smooth consistency, skin-friendly pH, and overall formulation stability.

The addition of Aloe vera and turmeric further improved the therapeutic potential of the polyherbal gel. Aloe vera provided moisturizing, soothing, and wound-healing benefits, while turmeric contributed antioxidant, antimicrobial, and anti-inflammatory activities because of the presence of curcuminoids. Together, these herbal ingredients offered a comprehensive approach for reducing bacterial growth, inflammation, oxidative stress, and skin pigmentation associated with acne.

Overall, the factorial design approach enabled systematic optimization of the polyherbal gel and helped identify the most effective combination of neem and liquorice extracts. The optimized formulation demonstrated

suitable stability and promising therapeutic potential for managing acne vulgaris and related hyperpigmentation. This herbal topical preparation may therefore serve as a safer alternative to conventional synthetic anti-acne treatments that are often associated with adverse effects. **Table no 5**

According to the table, we are using a **3^2 factorial design**, were

- **Factor A = Liquorice extract concentration**
- **Factor B = Neem extract concentration**
- Each factor has **3 levels**:
  - Low (-1)
  - Medium (0)
  - High (+1)

Table No. 5: Factorial Design of Formulation.

Formulation	Factor A	Factor B
F1	0.5%	1%
F2	0.5%	2%
F3	0.5%	3%
F4	1%	1%
F5	1%	2%
F6	1%	3%
F7	1.5%	1%
F8	1.5%	2%
F9	1.5%	3%

Formulation of Polyherbal Gel Containing Neem, Turmeric, Aloe vera, and Liquorice:

A polyherbal topical gel was developed using turmeric, aloe vera, neem, and liquorice extracts for the treatment of acne and associated hyperpigmentation. To obtain an optimized formulation, nine different batches (F1–F9) were prepared by systematically modifying the concentrations of neem and liquorice extracts while maintaining constant quantities of the remaining ingredients. Neem extract levels were adjusted to enhance antimicrobial activity against acne-related microorganisms, whereas liquorice extract concentrations were varied to improve depigmenting effects and reduce hyperpigmentation. The formulation strategy aimed to achieve an effective balance between anti-acne activity and skin-brightening potential.

Carbopol 934 was selected as the gelling polymer due to its ability to produce suitable viscosity and improve adhesion of the formulation to the skin. Aloe vera gel was incorporated for its moisturizing, soothing, and wound-healing properties. Turmeric extract was included because of its anti-inflammatory, antioxidant, antimicrobial, and tyrosinase inhibitory activities, which may help minimize acne lesions and post-inflammatory pigmentation. Neem extract contributed antibacterial activity against acne-causing microbes, while liquorice extract provided skin-lightening, anti-inflammatory, and depigmenting effects.

Propylene glycol was added as a penetration enhancer to facilitate better diffusion of the active constituents through the skin. Glycerin served as a humectant to maintain skin hydration, and methylparaben was incorporated as a preservative to protect the formulation from microbial growth. Triethanolamine was used to neutralize Carbopol 934 and adjust the pH, resulting in a smooth and stable gel structure. Purified water was added in sufficient quantity to obtain a final formulation weight of 100 g.

The prepared gel formulations were evaluated for several physicochemical characteristics, including appearance, homogeneity, pH, viscosity, spreadability, extrudability, washability, and stability. Comparative assessment of all batches was carried out to identify the optimized formulation with appropriate consistency, acceptable pH, improved spreadability, good skin applicability, and enhanced therapeutic performance for managing acne and hyperpigmentation**. Table no 6, In Figure no 11,12, and 13**

Table No. 6: Formulation of Polyherbal Gel Containing Neem, Turmeric, Aloe vera, and Liquorice.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Carbopol 934	1g	1g	1g	1g	1g	1g	1g	1g	1g
Neem	1g	2g	3g	1g	2g	3g	1g	2g	3g
Turmeric	1g	1g	1g	1g	1g	1g	1g	1g	1g
Liquorice	0.5g	0.5g	0.5g	1g	1g	1g	1.5g	1.5g	1.5g
Aloe vera	5g	5g	5g	5g	5g	5g	5g	5g	5g
Propylene Glycol	10g	10g	10g	10g	10g	10g	10g	10g	10g
Methyl Paraben	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g	0.2g
Triethanolamine	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml
Water	Q.s	Q.s	Q.s	Q.s	Q.s	Q.s	Q. s	Q. s	Q.s



Figure No. 11: Formulation of F1, F2, and F3.



Figure No. 12: Formulation of F4, F5, and F6.



Figure No. 13: Formulation of F7, F8, and F9.

PROCEDURE

The polyherbal gel formulations were developed using the dispersion technique with Carbopol 934 serving as the primary gelling polymer. The required amount of Carbopol 934 was gradually introduced into purified water under constant stirring to prevent agglomeration, followed by sufficient hydration to form a consistent gel matrix. Separately prepared methylparaben solution was incorporated as a preservative into the hydrated polymer system.

Propylene glycol and glycerine were subsequently mixed into the formulation to improve permeation and maintain moisture retention. Turmeric, Aloe vera, Neem, and Liquorice extracts were individually weighed and pre-dispersed in an appropriate solvent before being incorporated into the gel base. Multiple formulation batches were designed by modifying the concentrations of neem and liquorice extracts while maintaining all other components at fixed levels. Neem extract concentration was adjusted to study its influence on antimicrobial effectiveness against acne-associated microorganisms, whereas liquorice extract levels were optimized for depigmenting and skin-enhancing activity.

The herbal components were incorporated gradually with continuous mixing to ensure uniform distribution throughout the formulation. Neutralization of the Carbopol dispersion and pH adjustment were achieved by the slow addition of triethanolamine until the desired

gel consistency was obtained. Finally, purified water was added to achieve a total formulation weight of 100 g. The finished gel was mixed thoroughly to ensure stability and homogeneity before being packed into airtight containers for subsequent evaluation.

Evaluation of Gel

1. Physical appearance

Physical parameters include Colour, odor, Phase separation, Identification and consistency which can be checked visually. **In Figure no 11,12 and 13**

2. Homogenisty

It is a mixture which is completely uniform, identical, and consistent throughout its entire structure.

3. PH

The PH of (F1-F9) Formulation was measured by using a calibrated digital pH meter at a constant temperature. **In Figure no 14, 15.**



Figure No. 14: PH.



Figure No. 15: PH.

4. Spreadability

Spreadability of the formulation was assessed by placing about 1 g of the sample between two glass slides. A definite weight was placed on the upper slide for a few minutes to obtain a thin and uniform film of the formulation. The excess material was removed carefully, and the upper slide was allowed to move freely under the influence of an applied load. The time taken by the slide to move a specified distance was recorded, and the Spreadability was determined using the following formula: **In Figure no**

Spreadability (S) = M × L / T Where, M = weight tied to upper slide

L = length of glass slides, T = time taken to separate the slides

5. Extrudability

Extrudability is a significant evaluation parameter for semisolid dosage forms including gels, creams, ointments, and herbal formulations. It describes the ability of the formulation to come out smoothly from a tube when pressure is applied. This property is important because it reflects the ease with which the product can be handled and applied during use. A formulation with proper extrudability allows uniform dispensing without requiring excessive force, thereby improving patient convenience and acceptability.

To evaluate extrudability, the prepared formulation is packed into a clean collapsible tube and sealed carefully. A fixed amount of pressure or weight is then applied to the tube for a specified duration. The quantity of formulation expelled from the tube is collected and measured. The result provides information about the consistency and flow behavior of the preparation. A formulation that extrudes easily and uniformly is considered to possess good extrudability characteristics suitable for topical application.

6. Washability Test

The washability test is carried out to determine how easily a topical formulation can be removed from the skin after application. This parameter is important for products such as gels, creams, and ointments because it affects user comfort and convenience during regular use. A formulation with good washability can be cleaned from the applied surface using plain water without causing irritation or requiring excessive rubbing.

In this test, a small amount of the prepared formulation is spread evenly over the skin or a glass surface and kept for a short period. The applied area is then rinsed with water, and the ease of removal is observed visually. Formulations that are removed quickly and completely are considered to have satisfactory washability properties. This evaluation provides useful information regarding the practical usability and patient acceptability of the formulation.

7. Stability test

The stability study was performed to examine the behaviour of the prepared formulations during storage. Different parameters such as appearance, texture, pH, Odor, and consistency were checked at regular intervals to identify any changes occurring over time. The formulations were kept under appropriate storage conditions throughout the study period.

Among all the batches, formulations F1, F2, and F3 showed comparatively lower stability. During storage, these formulations became more acidic and slight changes in their physical characteristics were noticed. The reduction in pH indicated possible degradation or instability of certain formulation components.

Formulations F4, F5, and F6 remained stable throughout the observation period and showed the best overall performance. These batches retained their original color, consistency, and pH without noticeable deterioration. No evidence of separation, foul Odor, or contamination was observed, suggesting good stability of the formulations. In contrast, formulations F7, F8, and F9 developed an excessively thick consistency during storage. Their high viscosity may create favourable conditions for microbial contamination and can also affect ease of application. Due to these drawbacks, these formulations were considered less suitable for prolonged storage and regular use.

8. Antimicrobial Activity

The antimicrobial activity of the formulation intended for the treatment of acne and hyperpigmentation was evaluated by examining its effectiveness against acne-causing microorganisms such as *Cutibacterium acnes*, *Staphylococcus aureus*, and *Escherichia coli*. The assessment was carried out using the agar well diffusion technique. Sterile nutrient agar plates were prepared and inoculated with the selected bacterial cultures. Wells were then created in the agar medium with the help of a sterile cork borer, and a specific quantity of the formulation was added into each well. The plates were incubated at 37°C for 24 hours to allow microbial growth. After incubation, the antimicrobial potential of the formulation was determined by measuring the diameter of the clear zone formed around the wells, known as the zone of inhibition. A greater zone of inhibition indicated stronger antimicrobial effectiveness. This test helps to confirm the capability of the formulation to control microbial growth associated with acne, thereby supporting its role in reducing acne lesions and preventing post-inflammatory hyperpigmentation. **Table no 7.**[17]

Table No. 7: Evaluation of Gel

Formulation Batches	Consist-ency	PH	Extrud-ability	Spread-ability	Stability testing
F1	Semisolid	5.57	Well	5	Least
F2	Semisolid	5.58	Good	5.5	Least
F3	Semisolid	5.99	Good	6	Least
F4	Semisolid	5.7	Well	5.6	Least
F5	Semisolid	6	Well	5	Moderate
F6	Semisolid	6	Excellent	5	Excellent
F7	Semisolid	5.7	Good	5.5	Good
F8	Semisolid	5.59	Good	5	Good
F9	Semisolid	5.7	Good	6	Good

RESULT

The evaluation results indicated that all prepared polyherbal gel formulations (F1–F9) exhibited satisfactory physical and pharmaceutical properties. The developed gels showed uniform appearance, proper homogeneity, and smooth consistency without any visible phase separation. The pH of each formulation was observed to be slightly alkaline while remaining suitable for topical skin application. All batches demonstrated acceptable spreadability, washability, and ease of extrusion from the container.

Among the evaluated formulations, batch F6 was selected as the optimized formulation because it exhibited comparatively better pH, excellent extrudability, desirable consistency, and improved overall evaluation parameters. Formulations F4 and F7 also produced satisfactory results and showed good performance during evaluation studies; however, their characteristics were comparatively less favourable than formulation F6. Overall, the findings suggested that the formulated polyherbal gels possessed suitable properties for topical delivery and may be beneficial for the management of acne and associated hyperpigmentation.[21]

DISCUSSION

The prepared polyherbal gel formulations (F1–F9) containing turmeric, Aloe vera, neem, and liquorice were successfully evaluated for various formulation parameters. All batches exhibited acceptable physical characteristics such as uniform appearance, smooth consistency, good spreadability, and satisfactory extrusion behaviour. The pH of the formulations was slightly alkaline and remained appropriate for topical use.

Among the prepared batches, formulation F6 demonstrated comparatively better overall evaluation results, including suitable pH, improved consistency, and excellent extrudability, and was therefore selected as the optimized formulation. Formulations F4 and F7 also showed good performance during evaluation studies, although their results were slightly lower than F6. The combination of herbal ingredients may help provide beneficial effects against acne and associated hyperpigmentation due to their antimicrobial, anti-

inflammatory, antioxidant, and skin-soothing properties.[20]

CONCLUSION

The present investigation demonstrated the successful development of polyherbal gel formulations containing turmeric, Aloe vera, neem, and liquorice for topical management of acne and hyperpigmentation. All prepared batches (F1–F9) exhibited satisfactory formulation characteristics such as uniform consistency, good homogeneity, acceptable spreadability, and proper extrusion behaviour. The pH of all formulations remained slightly alkaline and appropriate for skin application.

Among the evaluated formulations, batch F6 showed comparatively superior overall characteristics when compared with F4 and F7. The formulation exhibited better consistency, suitable pH, and excellent extrudability during evaluation studies. Based on the obtained results, formulation F6 was selected as the optimized batch and may be considered a suitable herbal gel formulation for acne and post-inflammatory hyperpigmentation management.

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Conflicts Of Interest: Nil

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