ALPINIA GALANGA-INFUSED HERBAL GEL FOR ACNE VULGARIS

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Abstract

Acne vulgaris is a chronic skin condition affecting millions globally, often leading to physical discomfort and psychological distress. In search of safer, natural alternatives to conventional therapies, this study explores the therapeutic potential of *Alpinia galanga*, a medicinal plant known for its anti-inflammatory, antimicrobial, and antioxidant properties. A topical herbal gel infused with *Alpinia galanga* extract was formulated and evaluated for its efficacy in treating mild to moderate acne vulgaris.

The formulation underwent phytochemical analysis, stability testing, and many more evaluations on volunteers. findings suggest that *Alpinia galanga*-based gel offers a promising plant-based remedy for acne management, combining efficacy with skin compatibility. This research underscores the growing relevance of herbal therapeutics in dermatology and advocates further investigation into traditional botanicals for modern skincare applications.

Keywords: Acne Vulgaris, Alpinia Galangal, Herbal Gel, Anti-microbial Properties, Skin

1. Introduction

The skin, the body's largest organ, acts as a protective barrier against environmental factors, including UV light, pathogens, and toxins. It regulates temperature, prevents fluid loss, supports immune defense, and enables sensory perception. Adaptable in thickness and function, the skin comprises three layers: the epidermis, dermis, and hypodermis, each with distinct roles. The skin's complex structure forms the body's first barrier against pathogens, UV light, chemicals, and mechanical injury while also regulating temperature and water loss (1).

Structure of skin (2)

The skin, the body's largest organ, spans about 1.5–2 m^2 in adults and includes accessory structures such as glands, hair, and nails. It consists of three primary layers:

- **Epidermis**: The outermost layer, composed of stratified keratinized squamous epithelium, provides a protective barrier. It lacks blood vessels but receives nutrients from the dermis. Epidermal cells undergo continuous renewal, with full replacement occurring monthly.
- **Dermis**: A tough, elastic layer made of connective tissue, collagen, and elastic fibers. It contains blood vessels, nerves, sweat glands, sebaceous glands, and hair follicles, contributing to skin strength, elasticity, and sensory function.
- **Hypodermis**: The deepest layer, composed of adipose and connective tissue, provides insulation, energy storage, and cushioning while anchoring the skin to muscles and bones (3).

Sebaceous glands

Sebaceous glands are specialized skin organs that produce and secrete sebum, an oily substance that maintains skin hydration and forms a protective barrier. Most are associated with hair follicles, while some exist independently (4).

These glands contribute 90% of the skin's surface lipids and play a key role in skin homeostasis. They function through holocrine secretion, where sebocytes self-destruct to release sebum (5). Despite their epithelial origin, sebocytes also participate in lipid synthesis, making sebaceous glands a unique endocrine target. Sebum, rich in squalene and fatty acids, is essential for lubrication and defense against environmental and microbial threats (6).

Sebaceous glands develop early in fetal life, appearing between weeks 13–15 and forming the pilosebaceous unit with hair follicles. Sebum production begins at birth, peaks in infancy, declines, and surges again during adrenarche (around 9 years old) until late adolescence**.** While their number remains constant, gland size increases with age, especially during puberty (6).

Sebum

Sebum is a light yellow, viscous fluid produced by sebaceous glands, consisting of triglycerides, free fatty acids, squalene, wax and sterol esters, and free sterols (5). It plays a crucial role in maintaining the skin's moisture balance and is primarily regulated by androgens**,** with variations in production among individuals (7). Acne-prone individuals secrete more sebum, often with lower linoleic acid levels and increased oxidative stress, leading to inflammation (8). High-fat, high-carb diets, especially those with milk and hyperglycemic foods, may boost insulin and IGF-I, worsening acne. Conversely, caloric restriction reduces sebum, and low-glycemic diets may help regulate it (4) .

Acne occurs when excess sebum and dead skin cells clog follicles, fostering bacterial growth and inflammation. However, seborrhea alone doesn't cause acne, as treatments targeting inflammation - like antibiotics and retinoids - can be effective even without reducing sebum production (9).

Acne vulgaris

Acne vulgaris is a common skin disorder, especially during adolescence**,** affecting areas with dense sebaceous follicles like the face, chest, and back. It manifests as seborrhea (red, scaly skin), comedones (blackheads, whiteheads), papules, pustules, nodules, and cysts**,** sometimes leading to scarring**.** While not life-threatening, severe acne can impact psychological well-being and social interactions (10).

Pathophysiology of acne

Acne develops due to four key factors**:** excess sebum production**,** follicular hyperkeratinization**,** overgrowth of Propionibacterium acnes (P. acnes)**,** and inflammation (11)**.** Proper diagnosis and treatment require distinguishing between inflammatory lesions (papules, pustules, nodules, cysts) and non-inflammatory lesions (blackheads, whiteheads) (12).

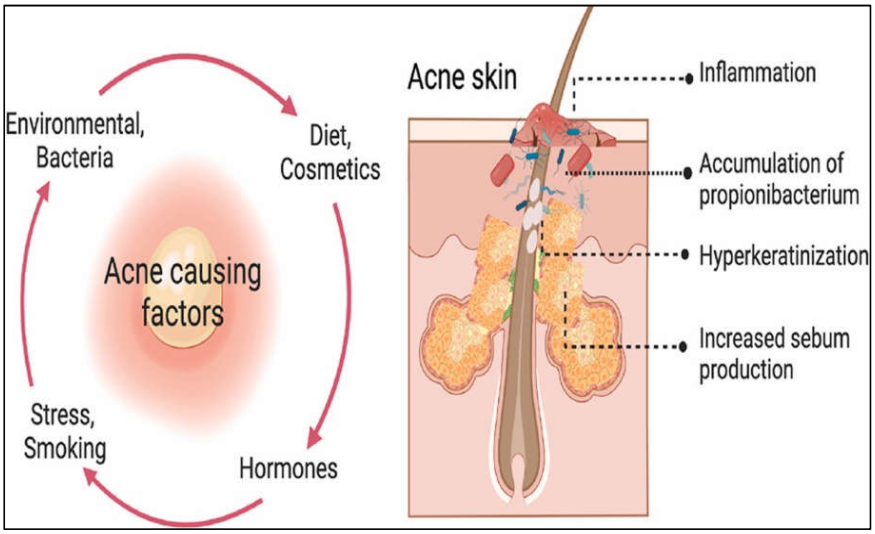

Figure 1.1. Pathophysiology of acne

Types of acne lesions

Acne manifests in various forms, including acne conglobata, acne rosacea, acne fulminans, acne cosmetica, acne excoriée (picker's acne), acne medicamentosa, acne chloracne, and acne mechanica (13). However, acne vulgaris is the most common type, accounting for 99% of all cases**.** It consists of two main lesion types

1. **Non-inflammatory lesions:** Open comedones **(**blackheads**)** and closed comedones **(**whiteheads**).**
2. **Inflammatory lesions:** Papules, pustules, nodules, and cysts (14).

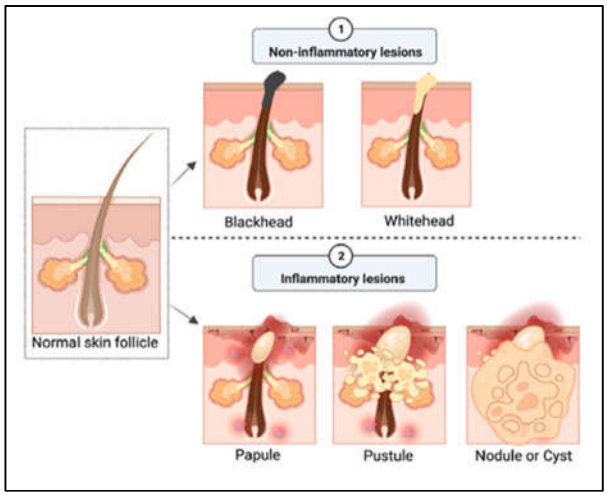

Figure 1.2. Types of acne lesions

Conventional acne treatments

Acne treatment aims to manage existing lesions by controlling sebum secretion, follicular hyperkeratinization, and propionibacterium acnes infection**.** The main approaches include

- **Medications**: Anti-inflammatory and antibacterial drugs, administered topically, orally, or systemically (12,15).
- **Physical Therapies**: Optical therapy, cryotherapy, comedone extraction, cryoslush therapy, and intralesional corticosteroids (12).
- **Combination Therapy**: A mix of topical and oral treatments is often more effective (16).

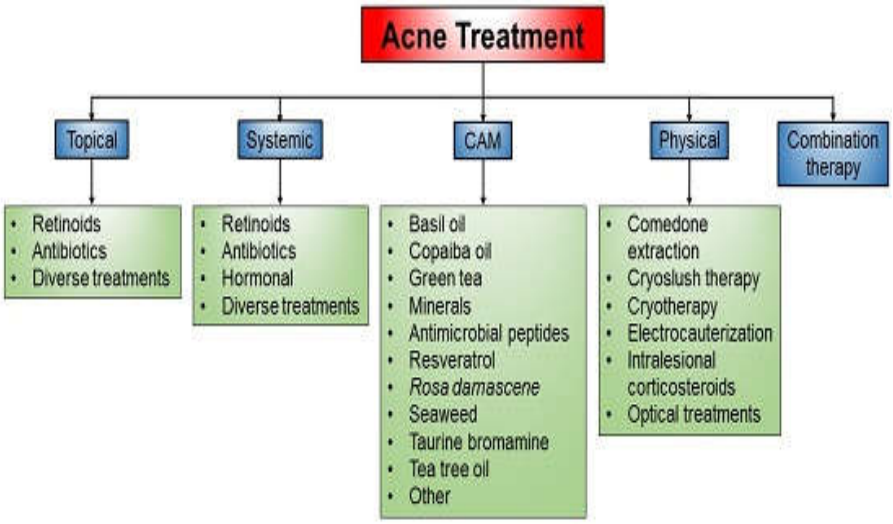

Figure 1.3. Different acne treatment

Synthetic polymer for acne treatments

Benefits

Synthetic treatments like retinoids (tretinoin, adapalene) and benzoyl peroxide effectively target acne by promoting cell turnover, reducing sebum, and eliminating bacteria**.** They offer faster results, undergo rigorous clinical testing, and come in various formulations (creams, gels, oral medications) for customized treatment (17).

Limitations

Potential side effects include skin irritation, redness, and dryness**.** Prolonged antibiotic use may lead to bacterial resistance, while hormonal therapies can cause mood changes and increased clot risk**.** Strong treatments like isotretinoin pose severe risks, including liver damage and birth defects (18)**.**

Due to these drawbacks, plant-based alternatives are being explored to combat antibiotic resistance and high treatment costs**.** Novel drug delivery systems targeting sebaceous glands could enhance treatment efficacy while minimizing side effects (18).

Natural products for acne treatment

Plants contain medicinal phytochemicals with antibacterial, antioxidant, anti-inflammatory, keratolytic, and sebum-regulating properties, making them effective in acne management (19). Organic acids derived from natural sources are commonly used as keratolytics in dermatology. Key plant-derived compounds beneficial for acne treatment include terpenes, terpenoids, flavonoids, alkaloids, phenolic compounds, saponins, tannins, and essential oils (20).

Advantages of natural acne treatments

1. **Gentle on Skin** – Causes less irritation compared to synthetic treatments (e.g., tea tree oil, aloe vera).
2. **Anti-Inflammatory** – Reduces redness and swelling (e.g., green tea extract, honey).
3. **Antibacterial Properties** – Targets Propionibacterium acnes (e.g., tea tree oil, neem oil).
4. **Rich in Antioxidants** – Protects skin from oxidative stress (e.g., turmeric, green tea).
5. **Hydration & Skin Repair** – Moisturizes and strengthens the skin barrier (e.g., honey, aloe vera) (21).

Alpinia galangal

India is well known for its rich heritage of medicinal plants used in treating various diseases, with green plants being a major source of bioactive compounds. *Alpinia galanga* (Linn) from the Zingiberaceae family is one such medicinally significant plant, widely cultivated in Indonesia and Malaysia. It is botanically known by various names, including Kulanjan, Pera-rattai, and Arattha. Naturally occurring in shady and marshy lands, it is predominantly cultivated in South and North India (22)**.**

The plant exhibits distinct morphological features such as its leaf apex, leaf base, rhizome, root, fruit, and seeds**.** Medicinally, *Alpinia galanga* possesses anti-inflammatory, antioxidant, antimicrobial, antiulcer, antiemetic, and antidiabetic properties. Its seeds act as cardiotonic and hypotonic agents**,** while the rhizome extract is used as a stomachic and carminative. Additionally, its tubers are beneficial in treating bronchitis, fever, and diabetes mellitus (23)**.** Commonly known as Greater galanga in English and Kulanjan in Hindi, *Alpinia galanga* is widely utilized in

Ayurveda and Siddha medicine across southern Indian regions, particularly for managing ailments like diabetes (24).

Morphology

Alpinia galanga (Greater galanga) has tuberous roots with a mild fragrance and oblong-lanceolate leaves that are sharp, glabrous, and green on top with a paler underside. The plant features short, rounded ligules, long glabrous scales, and white calloused sheaths. It produces 30 cm panicles densely packed with greenish-white flowers and ovate-lanceolate bracts. The tubular calyx has irregular three-toothed margins, while the oblong corolla lobes have a green claw, a white blade with reddish striations, and two subulate glands at the base and apex. The orange-red fruit, about the size of a small cherry, completes its distinct morphology (25).

Figure 1.4. *Alpinia galangal* rhizome

The rhizome of *Alpinia galanga* L. is traditionally used to alleviate toothache, muscular swelling, rheumatism, and abdominal pain. One of its most active compounds, Terpinen-4-ol, contributes to its medicinal properties. The plant is rich in saponins, terpenoids, phenolics, flavonoids, carbohydrates, alkaloids, glycosides, and phytosterols. Ethanolic extracts isolate galanga flavonoids, which exhibit antitumor, antifungal, antioxidant, hypoglycemic, gastroprotective, hypolipidemic, and anti-inflammatory activities (26).

Additionally, methanolic and aqueous extracts of *Alpinia galanga* have demonstrated strong free radical scavenging activity, superoxide anion scavenging, and metal chelating properties. The ethanolic extract has also been found effective in inhibiting α-glucosidase and amylase (in vitro), showcasing good antimicrobial activity (27).

While widely used as a culinary spice and herbal remedy in traditional medicine, recent research highlights its potential in skincare and cosmetic formulations, particularly for its antioxidant properties in creams and other skincare products (22).

NEED OF INVESTIGATION

The investigation is necessary to bridge the gap between traditional knowledge and modern consumer expectations, ensuring the herbal gel is safe, effective, and marketable. The need for an investigation into an herbal gel arises due to several critical factors that ensure its effectiveness, safety, and acceptance in the market. Currently, herbs are applied to the treatment of various diseases such as acute and chronic conditions and in the treatment of cardiovascular disease, for treating the various problems like depression, anxiety, inflammation and to boost immune system. Now days, 70% of the people preferring the use of herbal medicine instead of allopathic medicine due to various side effects.

Herbal therapy is a holistic approach that integrates emotional, mental, and spiritual aspects of well-being. Naturopathic treatments consider lifestyle, as well as emotional, mental, and spiritual factors. Unlike conventional drugs, the use of herbs typically does not result in “drug-like” actions or adverse effects**.** Unlike synthetic drugs, which are chemically engineered, herbal drugs consist of a diverse range of natural compounds that work together synergistically. This complexity allows them to target multiple aspects of a condition simultaneously. For instance, a single herb may possess anti-inflammatory, antioxidant, and immune-boosting qualities. The mechanism of action of herbal drugs is often multifaceted, owing to their complex chemical composition. Herbal drugs tend to act on multiple pathways and systems within the body, offering a holistic approach to treatment. For example, an herb used to treat inflammation may also have antioxidant and immune-modulating properties, addressing both the symptoms and underlying causes of the condition. Herbal drugs are generally perceived as safer than synthetic drugs due to their natural origin and the complexity of their chemical composition.

The chemical constituents which is present in the herb gives several actions like antibacterial, anti-inflammatory, antiviral, anti acne, antimalarial, analgesic, antioxidant, etc.

Alpinia galanga L. exhibits anti acne action which kills the bacteria caused acne present in the skin surface. For studying the various actions of herbs on various diseases, there is need of investigation of the herbal plants

3. Experimental work

3.1 Collection of *Alpinia galanga* rhizomes

For this study, rhizomes of greater galangal were procured from a farm in Alsand, Maharashtra. Other excipients and chemicals were obtained from Adarsh College of Pharmacy, Vita. All the chemicals and excipients used in this dissertation work were of analytical grade.

3.2 Preformulation study

3.2.1 Authentication

The rhizomes of *Alpinia galanga* L. Willd. were collected from the fields of Alsand (Vita), were identified and authenticated at Balwant College, Vita (Sangli).

3.2.2 Extraction (29)

Maceration is a popular and inexpensive extraction technique used for the extraction of different bioactive compounds from plant material. However, it has certain limitations such as low extraction yield, lower efficiency and use of large amounts of methanol as solvent. The process consists of grinding of rhizomes into smaller particles to increase the surface area for easy mixing with methanol and efficient extraction of compounds. The efficiency for the removal of bioactive compounds from the plant material depends on the type of methanol and type of plant material (30).

Figure 31. Extract and powder of *Alpinia galanga* L

3.2.3 Description

The extract of *Alpinia galanga* L. was evaluated for its appearance, nature, color, and odor.

3.2.4 Moisture Content

Place a clean, dry weighing dish with its lid open in a hot air oven and dry it. Cool the dish in a desiccator to room temperature and weigh it (denote this weight as *a*). Add approximately 3 g of the sample powder to the dish and weigh again (denote this weight as *b*). Place the loaded dish, with the lid open, in an oven maintained at 102 ± 2°C for 2 hours. Remove the dish, close the lid, cool it to room temperature in a desiccator, and weigh it (denote this weight as *c*). Continue drying the sample in the oven (with the lid open) at 102 ± 2°C for 1-hour intervals. After each drying period, repeat the cooling and weighing process as described above. Repeat this cycle until two successive weights (*c*) differ by not more than 0.5 mg, indicating a constant weight.

$$\%\boldsymbol{Moisture\ content} = \frac{\boldsymbol{Initial\ weight - Final\ weight}}{\boldsymbol{Initial\ weight}} \times \mathbf{100}$$

3.2.5 Preliminary phytochemical screening of powder

1. Test for Alkaloids

A 0.2 g quantity of the powdered sample was boiled with 5 mL of 2% hydrochloric acid on a steam bath. The mixture was filtered, and 1 mL portions of the resulting filtrate were transferred into four separate test tubes. Each 1 mL portion was then treated with 2 drops of a specific reagent to carry out preliminary phytochemical tests.

- **Dragendorff's Test:** Formation of a red precipitate indicates the presence of alkaloids.
- **Mayer's Test:** Formation of a creamy white precipitate indicates the presence of alkaloids.
- **Wagner's Test:** Formation of a reddish-brown precipitate indicates the presence of alkaloids.
- **Picric Acid Test (1%):** Formation of a yellow precipitate indicates the presence of alkaloids (31).

2. Test for Flavonoids

A 0.2 g quantity of powdered extract was heated with 10 mL of ethyl acetate in a boiling water bath for 3 minutes. The mixtures were filtered separately, and the resulting filtrates were used for the following test

- **Ammonia Test**

A 4 mL portion of the filtrate obtained from the powdered sample was shaken with 1 mL of 1% dilute ammonia solution. After allowing the layers to separate, the appearance of a yellow coloration in the ammonia layer indicated the presence of flavonoids.

- **Aluminum Chloride Test**:

4 mL portion of the filtrate from the powdered sample was mixed with 1 mL of 1% aluminum chloride solution and observed for color change. The development of a light yellow coloration or a yellow precipitate confirmed the presence of flavonoids.

3. Test for Phenols

- **Ferric Chloride Test**:

A portion of the filtrate obtained from the powdered extract was treated with 5% ferric chloride solution. The appearance of a deep blue or black coloration indicated the presence of phenolic compounds.

- **Liebermann's Test**:

A portion of the powdered extract was heated with sodium nitrite, followed by the addition of sulfuric acid diluted with water. An excess of dilute sodium hydroxide solution was then added. The formation of a deep red, green, or blue coloration indicated the presence of phenolic compounds.

4. Test for Glycosides

A 0.1 g quantity of each powdered extract was mixed with 5 mL of dilute sulfuric acid in a test tube and boiled in a water bath for 15 minutes. The mixture was then cooled and neutralized with 20% potassium hydroxide solution. Subsequently, 10 mL of a mixture containing equal parts of Fehling's solution A and B was added, and the solution was boiled for 5 minutes. The formation of a dense red precipitate indicated the presence of glycosides.

5. Test for Steroids

About 1ml stock solution was taken in test tube and dissolved with 2ml chloroform. Then an equal volume of concentrated sulphuric acid added slowly by the side of test tube to the mixture. The upper layer in test tube turns into red and sulphuric acid showed yellow with green fluorescence indicating the presence of steroid.

6. Test for Saponins

A 0.1 g quantity of each powdered extract was boiled with 5 mL of distilled water for 5 minutes. The mixture was filtered while still hot, and the resulting filtrates were used for the following tests

- **EmulsionTest:** To 1 mL of the hot aqueous filtrate, a few drops of olive oil were added. An additional two drops of olive oil were then introduced, and the mixture was shaken vigorously. The formation of a stable emulsion indicated the presence of saponins.
- **Frothing Test:** To 1 mL of the hot aqueous filtrate, 4 mL of distilled water was added. The mixture was shaken vigorously and allowed to stand. The appearance of a stable froth confirmed the presence of saponins.

7. Test for Tannins

A 2-gm quantity of each powder extract was boiled with 5 mL of 45% ethanol for 5 minutes. The mixtures were then cooled and filtered. The resulting filtrates were subjected to the following tests.

- **Lead Sub-acetate Test:** To 1 gm of each powder extract, 3 drops of lead sub-acetate solution were added. The formation of a cream-colored, gelatinous precipitate indicated the presence of tannins.
- **Ferric Chloride Test:** To 1gm of each powder extract a small amount of distilled water was added followed by 2 drops of ferric chloride solution. The appearance of a transient greenish to black coloration confirmed the presence of tannins**.**

8. Test for Proteins

A 0.1gm quantity of each powder extract was mixed with 5 mL of distilled water and allowed to stand for 3 hours. The mixture was then filtered. To 2 mL of the resulting filtrate, 0.1 mL of Millon's reagent was added. The solution was shaken gently and kept aside for observation. The appearance of a yellow precipitate indicated the presence of proteins**.**

- **Biuret Test**

  A 0.1 g quantity of each powdered extract was dissolved in 2 mL of distilled water and transferred into a test tube. To this, 5 drops of 1% hydrated copper sulfate solution were added, followed by 2 mL of 40% sodium hydroxide solution. The test tube was shaken vigorously to ensure thorough mixing. The appearance of a purple coloration indicated the presence of proteins, specifically two or more peptide bonds.

9. Test for Carbohydrate

A 0.1 g quantity of each power extract was shaken vigorously with distilled water and then filtered. To the resulting aqueous filtrate, a few drops of Molisch's reagent were added and the mixture was shaken well. Then, 1 mL of concentrated sulfuric acid was carefully added along the side of the test tube to form a separate layer beneath the aqueous solution. The formation of a brown ring at the interface indicated the presence of carbohydrates**.**

3.3 Formulation

Sr. No	Ingredient	Quantity	Role
1.	*Alpinia galanga* powder	1g	Antimicrobial activity
2.	Carbapol 934	1g	Gelling agent
3.	Methyl paraben	0.2g	Preservative
4.	Propyl paraben	0.1g	Preservative
5.	Propylene glycol	5ml	Humectant
6.	Triethanolamine	1.2ml	Stabilizer

3.4 Preparation of herbal gel

To prepare the herbal gel, 1 g of Carbopol 934 was dispersed in 50 mL of distilled water and allowed to swell for 30 minutes. The mixture was then stirred vigorously to ensure uniform dispersion and gel formation. Separately, 0.1 g of methyl paraben and 0.2 g of propyl paraben were dissolved in 5 mL of distilled water by heating gently on a water bath. After cooling, 5 mL of propylene glycol was added to this preservative solution. Next, 1 g of *Alpinia galanga* powder was incorporated into the above solution. The total volume was then adjusted to 100 mL by adding the remaining distilled water. Finally, this prepared solution was gradually added to the Carbopol 934 gel base with continuous stirring. Triethanolamine was added drop wise to adjust the pH to skin-friendly range of 6.8 to 7.0, and to achieve the desired gel consistency.

3.5 Preliminary phytochemical screening of gel

Same procedure like preliminary phytochemical screening of powder sample (Sample is prepared gel here)

3.6 Evaluation of herbal gel (32)

3.6.1 Physical Properties

The herbal gel formulation was evaluated for its color, odor, appearance, consistency, and physical state.

- **Color:** The color of the gel was assessed through visual examination under natural light.
- **Odor:** The lid of the sample container was opened, and the aroma was gently wafted towards the nose for 2–3 seconds. The odor was compared with that of a standard sample, and the observation was matched against the specification.
- **Consistency:** The consistency of the gel was evaluated by manually rubbing a small quantity between the fingers to assess texture and spreadability.
- **State:** The physical state of the gel (semi-solid, smooth, etc.) was determined by visual inspection.

3.6.2 Determination of pH

The pH meter was first calibrated using a standard buffer solution. Approximately 0.5 g of the gel was accurately weighed and dissolved in 50 mL of distilled water. The pH of the resulting solution was then measured using the calibrated pH meter.

3.6.3 Homogeneity

The formulations were tested for homogeneity through visual inspection and by tactile evaluation (touch) to ensure uniform distribution of the ingredients.

3.6.4 After Feel Effect

The emollient effect, slipperiness, and the amount of residue remaining after the application of a fixed quantity of the gel were evaluated to assess the formulation's skin feel and performance.

3.6.5 Irritancy Test

A specific area (1 cm × 2 cm) on the dorsal surface of the left hand was marked. The gel was applied to the designated area, and the time of application was recorded. The site was observed at regular intervals for up to 24 hours to check for signs of irritancy, erythema, and edema, and the results were documented accordingly (33).

3.6.6 Viscosity Measurement

The viscosity of the gel was determined using a Brookfield viscometer equipped with an L-4 spindle. The spindle was rotated at speeds ranging from 20 to 60 rpm in both ascending and descending order, with a 20 rpm increment or decrement every 60 seconds. All measurements were performed in triplicate to ensure accuracy and reproducibility (34).

3.6.7 Washability

Approximately 0.1 g of the gel was applied to the skin and washed off under running tap water using minimal mechanical force. Good washability was indicated by the easy removal of the gel from the application site, leaving behind a non-greasy surface.

3.6.8 Spreadability

An accurately weighed quantity (0.5 g) of the gel was placed within a pre-marked 7 cm diameter circle at the center of a glass slide. Another glass slide was gently placed over the gel, and a series of weights (100 g and 200 g) were applied on top of the upper slide for two minutes at room temperature (25 ± 2°C). The diameter (in cm) of the spread gel was measured in triplicate, and the mean spreadability ± standard deviation (SD) was calculated. Spreadability was evaluated initially and then monthly over a period of three months (34).

3.6.9 Phase Separation

The prepared gel was transferred into a suitable wide-mouthed container and set aside for storage. Phase separation between the oil and aqueous components was observed after 24 hours.

3.6.10 Stability study

Stability testing was conducted under various conditions to evaluate parameters such as nature, texture, color, odor, and overall appearance.

3.6.11 Antimicrobial activity of herbal gel

Antibiotic Assay Medium was prepared by dissolving peptone (9.4 g), yeast extract (4.7 g), beef extract (2.4 g), sodium chloride (10.0 g), dextrose (10.0 g), and agar (23.5 g) in 600 mL of purified water, heat to boiling, check and adjust pH to 6.1 ± 0.1 using 1 M NaOH or 1 M HCl if needed, and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Prepare sample solutions at 5 mg/mL and 10 mg/mL, vortex for 1-2 minutes, and use directly. For standard solutions, dissolve 1 mg of each sample in 1 mL DMSO, vortex, and use 100 µL for inoculation.

The test organisms used are Staphylococcus aureus and Escherichia coli. Streak a loopful of each organism on pre-incubated Nutrient Agar slants, incubate at 30-35°C for 24 hours, then inoculate in 3 mL of sterile saline, vortex, adjust OD to 60-70% at 530 nm, and store at 2-8°C. Inoculate 2 mL of S. aureus and E. coli suspensions separately into 200 mL of molten, cooled (40-45°C) medium, pour 15-20 mL into sterile Petri plates, cool at room temperature, refrigerate for 15-20 minutes, and ensure uniform thickness. Using an 8-10 mm stainless steel borer, make 4-5 agar

wells per plate, label for sample, standard, and negative control, and proceed with antimicrobial activity testing.

3.6.12 Anti acne activity of herbal gel

To evaluate anti-acne activity, *Propionibacterium acnes* is used as the test organism. A loopful is streaked on Nutrient Agar slants and incubated at 30–35°C for 24 hours. The growth is suspended in 3 mL of sterile saline, vortexed, and adjusted to 60–70% OD at 530 nm (approx. 10^6–10^7 CFU/mL), then stored at 2–8°C.

For analysis, 2 mL of this inoculum is mixed with 200 mL of molten Antibiotic Assay (40–45°C). About 15–20 mL of the inoculated medium is poured into Petri plates to a 3–4 mm depth, solidified at room temperature, and hardened in a refrigerator. Wells (8–10 mm) are made and filled with 100 µL each of the test sample, standard antibiotic, and negative control. Plates are incubated under appropriate anaerobic conditions at 30–35°C for 24–48 hours. Anti-acne activity is assessed by measuring the zone of inhibition around each well.

4. RESULT AND DISCUSSION

4.1 Preformulation study

4.1.1 Authentication of rhizomes

The *Alpinia galanga* L. specimens were authenticated at Balwant College, Vita, Sangli. Upon examination, the rhizomes were confirmed to belong to the Zingiberaceae family. The authentication was carried out by Dr. Shankar M. Shendage (M.Sc., Ph.D., M.B.A., FIAAT), Associate Professor, Department of Botany.

4.1.2 Description

The general appearance of powder, its nature, odour, and colour was found to be as follows in Table.

Table 4.1. General appearance of powder

Name of Powder	Colour	Odour	Appearance
Alpinia galanga L	Yellowish brown	Characteristic	Dry

4.1.3 Determination of moisture content

Moisture content of *Alpinia galanga L* willed rhizomes was found to be 2%.

4.1.4 Preliminary phytochemical screening

The preliminary phytochemical investigation was carried out for powder of *Alpinia galanga L* for the detection for various phyto-constituents by using standard procedure.To identify the constituents the results were recorded and detailed in below table (36) (37).

Table 4.2. Preliminary phytochemical screening of powder

Sr. No	Chemical Constituent	Result
1.	Alkaloids	Present
2.	Flavanoids	Present
3.	Phenols	Present
4.	Glycosides	Present
5.	Steroids and Terpenoids	Present
6.	Saponins	Present
7.	Tannin	Present
8.	Proteins	Present
9.	Carbohydrates	Present

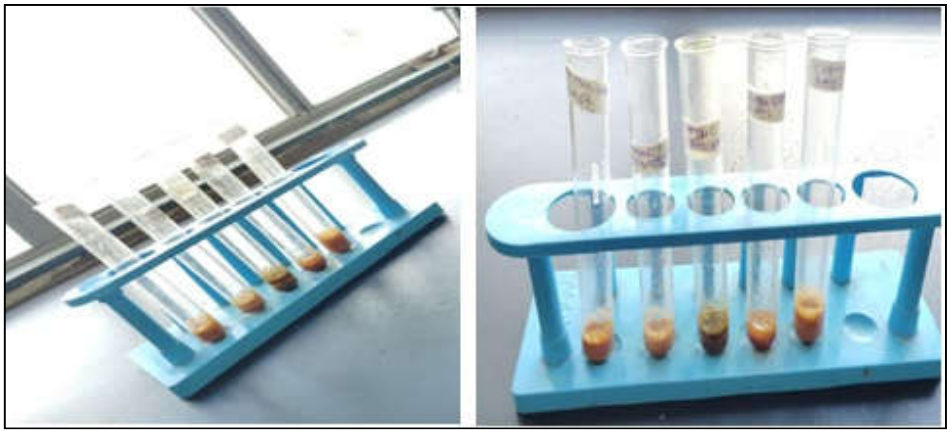

Figure 4.2. Phytochemical screening of powder

4.2 Evaluation of herbal gel

4.2.1 Physical properties

The formulated herbal gel was evaluated for its physical characteristics, including color, odor, consistency, and state as shown in Table 3. The gel exhibited a brownish-yellow color**,** which is

typical for formulations containing natural herbal extracts. It possessed a characteristic herbal odor, indicating the presence of aromatic phytoconstituents without any unpleasant or rancid smell. The consistency was smooth, uniform, and free from lumps or grittiness, which is desirable for easy application and good skin feel. The semisolid state of the gel was appropriate for topical application, ensuring good spreadability and adherence to the skin surface. These physical attributes reflect the acceptable organoleptic quality and aesthetic appeal of the herbal gel, which are crucial for patient compliance and consumer acceptance.

Table 4.3. Physical properties

Colour	Odour	Consistency	State
Brownish yellow	Characteristics	Smooth	Semisolid

1.2.2 Preliminary phytochemical screening

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7.	Tannins	Present
8.	Protein	Present
9.	Carbohydrates	Present

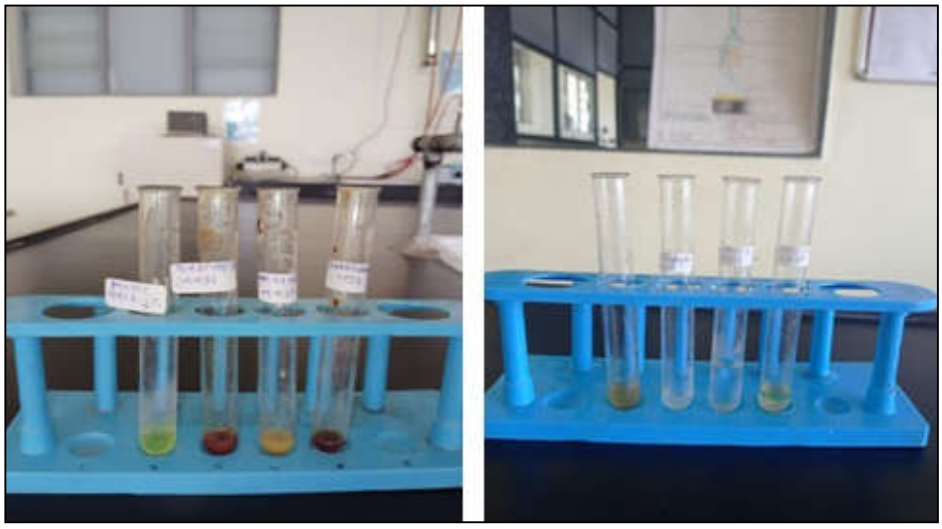

Figure 4.3. Phytochemical screening of herbal gel

4.2.3 Determination of pH

The pH meter was first calibrated using a standard buffer solution. Approximately 0.5g of the gel was accurately weighed and dissolved in 50 mL of distilled water. The pH of the resulting solution was then measured using the calibrated pH meter. The pH of the herbal gel was found to be 6.9. It was found to be neutral.

4.2.4 Homogeneity

The formulation was tested for homogeneity by visual appearance and by touch. According to the test the gel was homogeneous (38).

4.2.5 After feel effect

The herbal gel has a smooth, non-greasy texture that spreads effortlessly on the skin, leaving a light emollient layer that makes the skin feel soft and moisturized.

4.2.6 Irritancy test

The formulation was evaluated for irritancy by monitoring various parameters over a 24-hour period. No signs of irritancy, including edema (swelling) or erythema (redness), were observed, indicating that the formulation is non-irritating. The formulation's non-irritating nature further supports its potential for use in dermatological and cosmetic applications, where prolonged contact with the skin is common (39).

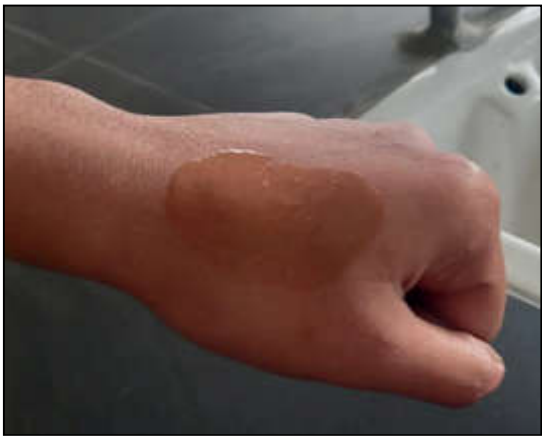

Figure 4.4. Irritancy test

4.2.7 Viscosity measurement

The viscosity of the formulated herbal gel was measured using a Brookfield viscometer (spindle No. 1.5) at 60 rpm and 25.4 °C. The recorded viscosity was 2990 cP, indicating that the formulation possesses a suitable semi-solid consistency. The observed viscosity value of 2990 cP suggests that the gel has an appropriate flow behaviour neither too thick nor too runny ensuring ease of application and sufficient retention on the skin (38).

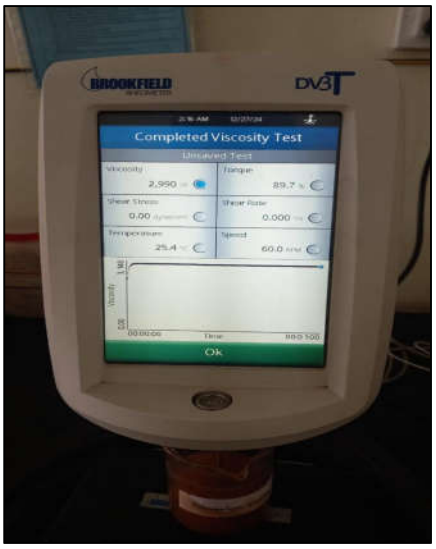

Figure 4.5. Viscosity measurement

4.2.8 Washability

The herbal gel formulation exhibited excellent washability. Upon rinsing with tap water, the gel was easily removed from the skin surface without requiring excessive rubbing. The effortless removal of the gel upon rinsing indicates its non-greasy, non-adherent nature, which enhances user

comfort during and after application. Making it suitable for repeated use without causing skin irritation (40).

Figure 4.6. Washability

4.2.9 Spreadability

Spreadability is a critical attribute for topical formulations, influencing the ease with which a product can be applied over the skin surface. The observed shorter slide separation time indicates that the herbal gel possesses a low resistance to spreading, which enhances user comfort and promotes uniform application over the affected area (41).

Figure 4.7. Spreadability

4.2.10 Phase separation

After a 24-hour observation period, no phase separation was observed in the formulated herbal gel. The gel remained homogeneous with no visible changes in its appearance or consistency. The resistance to phase separation suggests that the gel is not prone to settling or demixing, which is crucial for maintaining the efficacy and consistency of topical applications.

4.2.11 Stability studies

Stability testing of all formulated batches was conducted under various conditions, including room temperature, cool temperature, and warm temperature. The nature, color, and odor of the herbal gel were monitored over a defined period. No significant changes were observed in any of these parameters. The gel exhibited the highest stability when stored at cool temperatures (40).

Table 4.5. Stability studies

Sr. No	Parameter	Initial studies	Room temperature	Cool temperature	Warm condition
1.	Nature	Sticky	No change	No change	No change
2.	Colour	Brownish yellow	No change	No change	No change
3.	Odour	Characteristic	No change	No change	No change
4.	Texture	Smooth	No change	No change	No change

Figure 4.8. Stability studies of herbal gel

4.2.12 Antimicrobial activity of herbal gel

The antimicrobial activity of the formulated herbal gel was evaluated against two bacterial strains: Staphylococcus aureus and Escherichia coli using the agar well diffusion method. Gentamicin (1 mg/mL) served as the standard and exhibited large zones of inhibition 36 mm against S. aureus and 35 mm against E. coli. Sample A (marketed formulation) at 1 mg/mL showed a zone of

inhibition of 18 mm against S. aureus and 2 mm against E. coli. Sample B (herbal formulation) at 1 mg/mL showed a zone of 6 mm against S. aureus and 2 mm against E. coli and no inhibition was observed in the control.

The results indicate that both Sample A and Sample B exhibit antibacterial activity, with greater efficacy against *S. aureus* than *E. coli*. Sample A was more potent than Sample B, particularly against the gram-positive strain. The limited activity against *E. coli* may be due to the protective outer membrane characteristic of gram-negative bacteria, which reduces permeability to many antimicrobial agents.

Although the herbal gel showed its effectiveness against *S. aureus* suggests potential as a natural antibacterial agent for treating gram-positive infections. With further optimization in concentration or composition, the herbal formulation could be enhanced for broader-spectrum antimicrobial efficacy.

Table 4.6. Antimicrobial activity of herbal gel

Sr. No	**Sample**	**Concentration (mg)**	**Zone of Inhibition *S.aureus* (mm)**	**Zone of Inhibition *S.aureus* (mm)**
1.	Control	-	-	-
2.	Standard Gentamicin	1 mg	36	35
3.	Sample A (Marketed formulation)	1 mg/ml	18	2
4.	Sample B	1 mg/ml	06	2

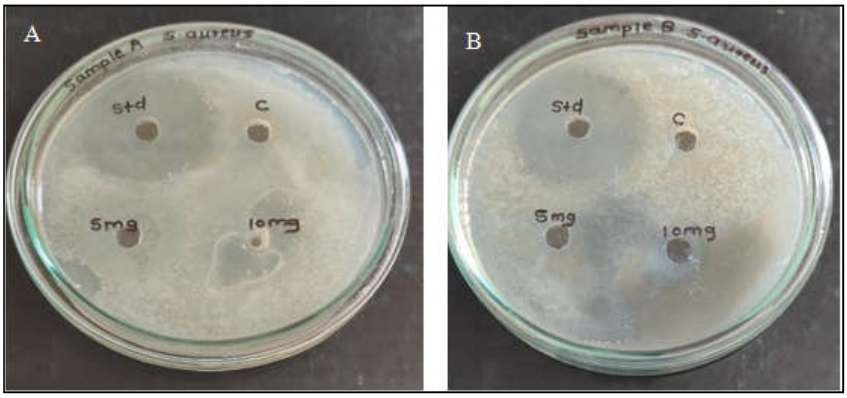

Figure 4.9. Antimicrobial activity of herbal gel on *Staphylococcus aureas*

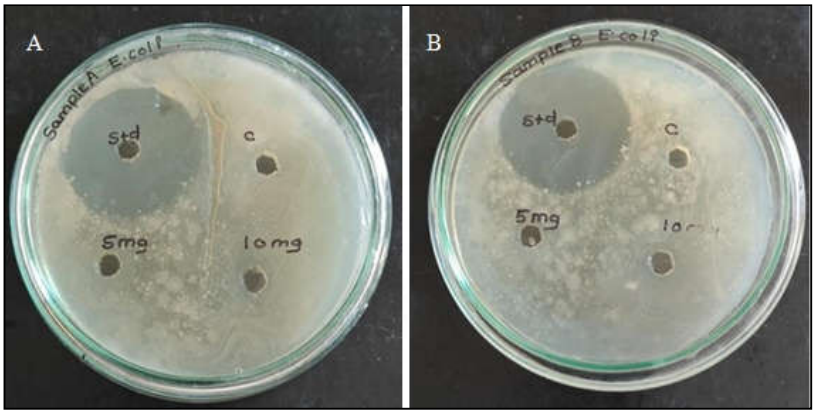

Figure 4.10. Antimicrobial activity of herbal gel on *E. coli*

4.2.13 Anti-acne activity of herbal gel

The anti-acne activity of the formulated herbal gel (Sample C) was assessed against Propionibacterium acnes using the agar well diffusion method. The marketed formulation (Kojivit, 10 mg/mL) was used as the standard and showed a zone of inhibition of 30 mm. Sample C exhibited a zone of inhibition of 6 mm at 5 mg/mL concentration and 12 mm at 10 mg/mL. No zone of inhibition was observed for the control.

Although the activity was lower compared to the standard marketed product, the dose-dependent increase in the zone of inhibition indicates that the herbal formulation possesses measurable antibacterial potential against acne-causing bacteria. The observed antimicrobial effect may be attributed to the presence of bioactive herbal components with known antibacterial or anti-inflammatory properties. The absence of any zone in the control group confirms that the observed activity is solely due to the active ingredients in the gel. These findings suggest that with further

optimization or combination with other herbal actives, the formulation can be a promising natural alternative for managing acne conditions with minimal side effects.

Table 4.7. Anti-acne activity of herbal gel

Sr. No	Sample	Concentration	Zone of Inhibition P.acne (mm)
1.	Control	-	-
2.	Standard Marketed formulation (Kojivit)	10 mg/ml	30
3.	Sample	5 mg/ml	06
		10 mg/ml	12

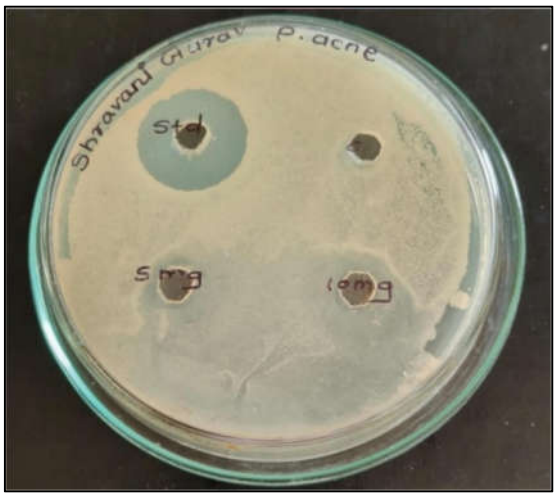

Figure 4.11. Antiacne activity of herbal gel on *P.acne*

5. CONCLUSION

Pre-formulation studies of *Alpinia galanga* L. were successfully conducted, resulting in the material being converted into a fine powder. This powdered form was then subjected to moisture content analysis, which revealed a moisture level of 2%. Subsequently, a preliminary phytochemical analysis was carried out. Extraction of *Alpinia galanga* L. was performed using a suitable solvent, and the resulting extract was further evaluated.

The anti-acne gel prepared was evaluated for various parameters such as physical appearance, homogeneity, odor, spreadability, loss on drying, irritancy, washability, antimicrobial activity, and stability.

The present research was undertaken for the design and development of an anti-acne gel formulated with *Alpinia galanga* L. extract, known for its anti-acne properties. *Alpinia galanga* L. has shown significant therapeutic potential in the treatment of various ailments, including colds, pain, inflammation, and microbial infections. It is also recognized as a valuable source of antioxidants that contribute to overall human health. Herbal remedies, such as those derived from *Alpinia galanga* L., are widely accepted due to their perceived safety and minimal side effects compared to synthetic drugs. Globally, over 80% of the population relies on medicinal plants as a primary source of healthcare.

Many of these plants have demonstrated the ability to alleviate symptoms, prevent disease complications, promote cellular regeneration, and combat pathogenic microorganisms. Based on the results of this study, it can be concluded that the formulation and evaluation of the gel containing the natural extract of *Alpinia galanga* L. were successfully carried out. The extract demonstrated notable antimicrobial properties. These in vitro findings indicate that the rhizome extract of *Alpinia galanga* L. is a promising natural source of antimicrobial agents, which could play a significant role in acne prevention.

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