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Development And In Vitro Evaluation Of Anti-Acne Gel Containing *Psidium Guajava* Leaf Extract

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ABSTRACT

Acne vulgaris is a common chronic inflammatory skin disorder affecting the pilosebaceous unit and is associated with microbial infection, excess sebum production, and inflammation. The present study focuses on the development and in vitro evaluation of an anti-acne gel formulated using *Psidium guajava* (guava) leaf extract, known for its antimicrobial, antioxidant, and anti-inflammatory properties.

The guava leaves were collected, authenticated, shade-dried, and subjected to hydroalcoholic extraction using the maceration technique. Preliminary phytochemical screening confirmed the presence of bioactive constituents such as flavonoids, tannins, and phenolic compounds. The extract was incorporated into a gel base using Carbopol 934 to prepare three formulations (F1, F2, and F3) with varying concentrations of the extract.

The formulated gels were evaluated for physicochemical parameters including physical appearance, pH, spreadability, viscosity, washability, and stability. All formulations showed acceptable organoleptic properties, with pH in the range suitable for skin application (6.0–7.0). Spreadability and viscosity studies indicated that formulation F1 exhibited optimal performance with good consistency and ease of application. Stability studies revealed that F1 remained stable under both room temperature and refrigerated conditions, while F2 and F3 showed moderate variations.

The antimicrobial activity was evaluated against *Cutibacterium acnes* using the agar well diffusion method. The formulation exhibited minimal antibacterial activity compared to the standard drug, indicating the need for further optimization.

In conclusion, the study demonstrates that *Psidium guajava* leaf extract can be successfully formulated into a topical gel with acceptable physicochemical properties, though enhancement of antimicrobial efficacy is required for improved therapeutic potential.

KEYWORDS: Anti-acne gel, *Psidium guajava*, Guava leaf extract, Acne vulgaris, Carbopol 934, Herbal formulation, Antimicrobial activity, *Cutibacterium acnes*, Topical drug delivery, Phytochemical screening

INTRODUCTION

The skin is the largest organ of the human body and acts as a primary protective barrier against environmental, chemical, and microbial insults[1]. It plays a vital role in maintaining physiological homeostasis, regulating body temperature, preventing excessive water loss, and providing immune defense[2]. Structurally, the skin consists of three major layers: the epidermis, dermis, and hypodermis. Embedded within these layers are specialized appendages such as hair follicles and sebaceous glands, collectively known as the pilosebaceous unit[3]. Dysfunction of this unit is closely associated with several dermatological conditions, among which acne vulgaris is one of the most common[4].

Acne is a widespread chronic inflammatory skin disorder affecting the pilosebaceous unit, which consists of hair follicles connected to sebum-producing glands[5]. The clinical manifestations of acne include seborrhoea (excess oil production), non-inflammatory lesions such as open (blackheads) and closed comedones (whiteheads), and inflammatory lesions including papules, pustules, nodules, and cysts[6]. In severe cases, acne may lead to permanent scarring and post-inflammatory hyperpigmentation. Acne commonly appears in areas where sebaceous glands are most concentrated, such as the face, neck, upper chest, shoulders, and back[7]. Although it predominantly affects adolescents due to hormonal changes, it can persist into adulthood and significantly impact psychological well-being, leading to reduced self-esteem and quality of life[8].

The pathogenesis of acne vulgaris is multifactorial and involves four major mechanisms. Firstly, there is excessive proliferation and abnormal shedding of keratinocytes, resulting in blockage of the follicular duct[9]. Secondly, androgen-induced hypersecretion of sebum creates an oily environment favorable for microbial growth[10]. Thirdly, there is colonization and proliferation of the bacterium *Cutibacterium acnes* (formerly *Propionibacterium acnes*) within the clogged follicles[11]. Finally, an inflammatory response is triggered due to bacterial metabolites and immune reactions, leading to redness, swelling, and lesion formation. These interconnected processes contribute to the initiation and progression of acne[12].

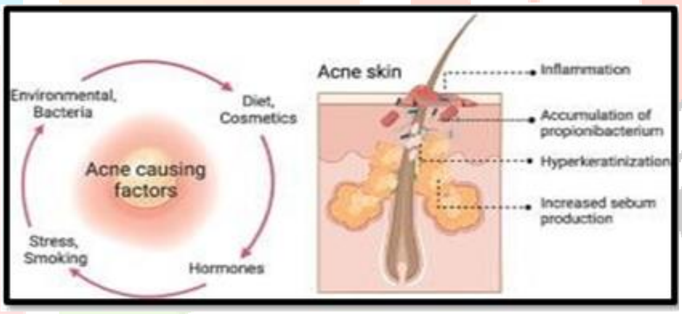

Figure 1: Pathogenesis of Acne & Contributing Factor

Management of acne depends on its severity and site of occurrence. Conventional treatments include topical and systemic therapies such as antibiotics, retinoids, and keratolytic agents. Among these, benzoyl peroxide is one of the most widely used topical agents due to its comedolytic, antibacterial, and anti-inflammatory properties against *Cutibacterium acnes*[13].However, prolonged use of synthetic drugs is often associated with adverse effects such as skin irritation, dryness, erythema, and the development of microbial resistance. These limitations highlight the need for safer and more effective alternative therapies[14].

Natural products derived from plant, animal, and mineral sources have long been used in traditional medicine for the treatment of various diseases. It is estimated that nearly 80% of the global population relies on herbal medicines for primary healthcare. Herbal formulations are gaining increasing attention due to their lower toxicity, reduced side effects, cost-effectiveness, and therapeutic efficacy. Unlike conventional treatments that primarily target symptoms, natural therapies often aim to address the underlying causes of disease, including inflammation and microbial imbalance[15].

Psidium guajava, commonly known as guava, belongs to the family Myrtaceae and is widely cultivated in tropical and subtropical regions[16]. It is an important medicinal plant with various therapeutic applications. Different parts of the plant, including leaves, bark, roots, and fruits, have been traditionally used to treat conditions such as diabetes, gastrointestinal disorders, infections, and skin diseases[17]. Guava leaves are particularly rich in bioactive phytoconstituents such as flavonoids, tannins, saponins, phenolic compounds, eugenol, and triterpenoids[18]. Among these, flavonoids and tannins are predominant and are known for their potent antimicrobial, antioxidant, and anti-inflammatory activities. These properties make *Psidium guajava* leaf extract a promising candidate for the treatment of acne vulgaris[19].

However, the direct application of plant extracts on the skin may be limited by poor absorption, instability, and reduced efficacy[20]. Therefore, incorporation into suitable topical delivery systems is essential to enhance therapeutic effectiveness[21]. Gels are one of the most preferred topical dosage forms due to their non-greasy nature, ease of application, good spreadability, and patient acceptability[22]. They form a thin film upon application, provide a cooling effect, and facilitate better penetration of active constituents into the skin[23].

Hence, the present study aims to develop and evaluate an anti-acne gel formulation containing *Psidium guajava* leaf extract. The study involves extraction of the plant material, phytochemical screening, formulation development, and evaluation of physicochemical properties and antimicrobial activity. This approach is intended to assess the potential of the herbal gel as a safe, effective, and economical alternative for the management of acne vulgaris.

MATERIALS AND METHODS

1. Collection and Authentication of Plant Material

Fresh, healthy, and disease-free leaves of *Psidium guajava* were collected from the local region during the appropriate growing season to ensure maximum phytoconstituent content. The collected plant material was carefully selected to avoid contamination, damaged parts, or microbial infection. The leaves were thoroughly washed with distilled water to remove adhering dust, soil particles, and other extraneous matter.

The cleaned plant material was then subjected to botanical identification and authentication by a qualified taxonomist from the Department of Botany, Smt. Kusumtai Rajarambapu Patil Kanya Mahavidyalaya, Islampur. Authentication was carried out based on morphological and taxonomical characteristics such as leaf shape, venation pattern, texture, and arrangement. A voucher specimen (TSN 29) was prepared, labeled, and preserved in the herbarium for future reference and reproducibility of the study.

Table1: Pharmacognostic Profile of *Psidium guajava*

Parameter	**Description**
Biological Name	*Psidium guajava*
Family	Myrtaceae
Common Name	Guava
Synonyms	Tropical apple
Part Used	Leaves
Geographical Source	Widely distributed in tropical and subtropical regions (India, Indonesia, Brazil, Bangladesh)
Plant Type	Small tree or shrub
Leaf Characteristics	Opposite, simple, oval-shaped, with prominent veins and aromatic odor
Phytoconstituents	Flavonoids, tannins, saponins, phenolics, eugenol, triterpenoids
Major Active Compounds	Quercetin, guaijaverin, gallic acid
Pharmacological Activities	Antimicrobial, anti-inflammatory, antioxidant, anti-diabetic

Traditional Uses	Treatment of diarrhea, wounds, infections, and skin disorders
Mechanism in Acne	Inhibits *Cutibacterium acnes*, reduces inflammation, controls sebum
Dosage Form Used	Topical gel formulation

2. Preparation of Plant Material

The authenticated leaves were shade-dried under controlled environmental conditions at room temperature (25–30°C) for 7–10 days to prevent degradation of thermolabile phytoconstituents. Direct sunlight was avoided to preserve the chemical integrity of active compounds such as flavonoids and phenolics.

After complete drying, the leaves were coarsely powdered using a mechanical grinder. The powdered material was passed through a sieve to obtain uniform particle size and stored in an airtight container, protected from moisture, light, and contamination until further processing.

3. Extraction of Plant Material

Extraction of phytoconstituents was carried out using the maceration technique with 70% ethanol as a hydroalcoholic solvent. The powdered plant material was soaked in a sufficient quantity of solvent in a closed container and allowed to stand for 48–72 hours with occasional shaking to enhance solvent penetration and extraction efficiency.The hydroalcoholic solvent was selected due to its ability to extract both polar and moderately non-polar compounds such as flavonoids, tannins, and phenolic constituents. After completion of maceration, the extract was filtered using Whatman filter paper to remove insoluble residues.

The filtrate was concentrated using a water bath maintained at 40–60°C to evaporate the solvent and obtain a semi-solid crude extract. This controlled evaporation ensured the preservation of heat-sensitive bioactive compounds.

Figure 2: Maceration Extraction Technique

4. Storage of Extract

The concentrated extract was transferred into a clean, dry, amber-colored airtight container to protect it from light-induced degradation. It was stored under refrigerated conditions (2–8°C) to maintain stability and prevent microbial growth. The extract was used for further phytochemical screening and formulation studies.

5. Phytochemical Screening

Preliminary qualitative phytochemical analysis of the extract was performed using standard procedures to identify the presence of major bioactive constituents such as alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds. These phytoconstituents are known for their antimicrobial, antioxidant, and anti-inflammatory properties, which contribute to anti-acne activity.

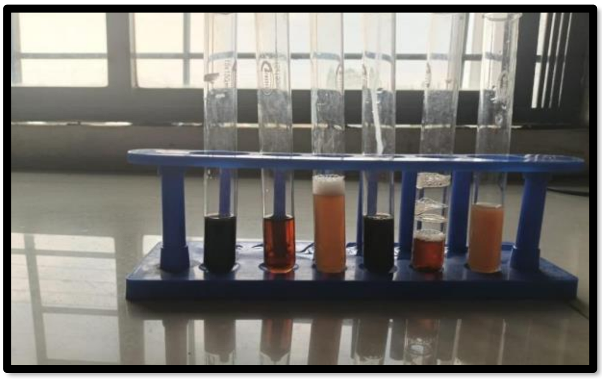

Figure 3: Phytochemical Screening Test

6. Formulation of Anti-Acne Gel

The anti-acne gel was formulated using Carbopol 934 as a gelling agent. Carbopol was dispersed in distilled water with continuous stirring and allowed to hydrate completely. The plant extract was dissolved in propylene glycol to enhance solubility and skin penetration. Methyl paraben was added as a preservative.

The extract solution was gradually incorporated into the hydrated Carbopol base with continuous stirring to ensure uniform distribution. Triethanolamine was added dropwise to neutralize the formulation and adjust the pH, resulting in the formation of a clear, homogeneous gel. The prepared gel was stored in suitable containers for further evaluation.

Table 2: Composition of Anti-Acne Gel

Sr No.	Ingredient	F1 (g)	F2 (g)	F3 (g)	Role
1	Guava Leaf Extract	0.20	0.40	0.60	Active Ingredient
2	Carbopol 934	0.10	0.10	0.10	Gelling Agent
3	Propylene Glycol	2.00	2.00	2.00	Humectant
4	Methyl Paraben	0.04	0.04	0.04	Preservative
5	Triethanolamine	q. s	q. s	q. s	Neutralizer
6	Distilled Water	q. s	q. s	q. s	Vehicle

Procedure:

The anti-acne gel was formulated using the Carbopol gel base technique. A precisely weighed amount of Carbopol 934 was gradually added to distilled water with constant stirring for about 30 minutes to ensure uniform dispersion and to prevent the formation of lumps. The mixture was then kept aside to allow complete hydration of the polymer.

In a separate container, the required quantity of *Psidium guajava* leaf extract was dissolved in a small volume of distilled water or propylene glycol. To this solution, methyl paraben was incorporated as a preservative.

The extract solution was then slowly added to the hydrated Carbopol base with continuous stirring to obtain a uniform and homogeneous mixture. Propylene glycol was also included as a humectant to enhance the moisturizing effect of the formulation.

Finally, triethanolamine was added dropwise to neutralize the formulation and adjust the pH, leading to the formation of a transparent and smooth gel. The final gel was transferred into a clean, airtight container and stored at room temperature for further evaluation studies.

ANTIMICROBIAL STUDY:

1.Equipment and Instruments

The antimicrobial study was carried out using standard laboratory instruments to ensure accuracy and reproducibility of results. The instruments used are listed below:

Table 3: Equipment and Instruments Used

Sr. No.	Instrument/Equipment
1	Analytical Balance
2	Vernier Caliper
3	Water Bath
4	Incubator (20–25°C)
5	Laminar Air Flow Chamber
6	Incubator (30–35°C)
7	Colorimeter
8	Refrigerator (2–8°C)
9	Zone Reader
10	Cyclomixer
11	Sonicator
12	Micropipettes

2. Media and Reagent Preparation

Table 4: Composition of Antibiotic Assay Medium No. 19

Ingredient	Quantity
Peptone	9.4 g
Yeast Extract	4.7 g
Beef Extract	2.4 g
Sodium Chloride	10.0 g
Dextrose	10.0 g
Agar	23.5 g
Distilled Water	1000 mL
Final pH (after sterilization)	6.1 ± 0.1

Antibiotic Assay Medium No. 19 was prepared for the antimicrobial study, maintaining a pH of 6.1 ± 0.2. The medium was prepared by dissolving all ingredients in purified water with continuous heating and stirring until completely dissolved. The pH was checked and adjusted using 1 M sodium hydroxide or 1 M hydrochloric acid if required. The prepared medium was distributed into suitable containers and sterilized by autoclaving at 121°C (15 lbs pressure) for 15 minutes.

3. Preparation of Sample Solution

The test samples (gel formulation) were prepared in concentrations of 5 mg/mL and 10 mg/mL using a suitable solvent for antimicrobial evaluation.

4. Preparation of Standard Solution

The standard solution was prepared by accurately weighing 1 mg of standard drug and dissolving it in 1 mL of DMSO in a volumetric flask. The solution was vortexed for 1–2 minutes to ensure complete dissolution. A volume of 100 µL of this solution was used for inoculation.

5. Preparation of Test Organism and Inoculum

The antimicrobial activity of the formulated gel was evaluated against *Cutibacterium acnes* (ATCC No. 11827). The stock culture of the microorganism was first activated by streaking a loopful onto pre-sterilized nutrient agar slants, followed by incubation at 30–35°C for 24 hours to obtain fresh and active growth. After incubation, a portion of the bacterial growth was aseptically transferred into 3 mL of sterile saline solution and vortexed thoroughly to prepare a uniform microbial suspension.

The turbidity of the suspension was then adjusted to approximately 60–70% optical density at 530 nm using a colorimeter to ensure a standardized inoculum concentration. This adjustment corresponds to an approximate viable count of 10^6–10^7 CFU/mL, which is suitable for antimicrobial testing. The prepared inoculum was stored under refrigerated conditions (2–8°C) and used for further experimental procedures.

6. Plate Preparation

Sterile molten Antibiotic Assay Medium No. 19 was allowed to cool to a temperature of 40–45°C to prevent thermal damage to the microbial inoculum. To this, 2 mL of the prepared microbial suspension of *Cutibacterium acnes* was added to 200 mL of the medium and mixed thoroughly to ensure uniform distribution of the organism throughout the agar.

Approximately 15–20 mL of the inoculated medium was then aseptically poured into sterile Petri plates to obtain a uniform agar layer with a thickness of about 3–4 mm. The plates were allowed to solidify at room temperature on a level surface and subsequently placed in a refrigerator for 15–20 minutes to ensure proper setting and hardening of the medium.

After solidification, wells of approximately 6 mm diameter were aseptically created in the agar using a sterile stainless steel borer. Each plate was prepared with 4–5 wells and appropriately labeled for standard, test samples, and control to facilitate accurate identification during analysis.

7.Antimicrobial Analysis (Agar Well Diffusion Method)

The antimicrobial activity of the formulated gel was evaluated using the agar well diffusion method against *Cutibacterium acnes*. A fixed volume of 100 µL of each prepared solution was carefully introduced into the respective wells of the inoculated agar plates. The test included a standard solution (1 mg/mL), test samples at concentrations of 5 mg/mL and 10 mg/mL, and a negative control consisting of dimethyl sulfoxide (DMSO).

Following the addition of the samples, the plates were maintained at refrigerated conditions (2–8°C) for 15–20 minutes to allow adequate pre-diffusion of the test solutions into the agar medium. This step ensured uniform distribution of the compounds before microbial growth commenced. The plates were then incubated at 30–35°C for a period of 24–48 hours under suitable conditions for bacterial growth.

After completion of the incubation period, the antimicrobial activity was assessed by measuring the diameter of the zones of inhibition formed around each well. The measurements were recorded in millimeters using a zone reader or Vernier caliper. The extent of inhibition was used as an indicator of the antimicrobial efficacy of the formulated gel in comparison with the standard.

RESULTS & DISCUSSION

Evaluation of Anti-Acne Gel

1. Physical Appearance

Table 5: Organoleptic Evaluation of Anti-Acne Gel

Parameter	Observation
Colour	Dark Brown
Odour	Characteristic Herbal
Consistency	Semi-solid
Smoothness	Smooth, no roughness
Homogeneity	Homogeneous

The formulated anti-acne gel exhibited a dark brown colour, which can be attributed to the presence of *Psidium guajava* leaf extract containing natural phytoconstituents such as tannins and flavonoids. The formulation showed a characteristic herbal odour, confirming the incorporation of plant-based ingredients. The semi-solid consistency indicates appropriate gel formation using Carbopol 934, making it suitable for topical application. Additionally, the smooth texture without any grittiness or phase separation suggests uniform dispersion of ingredients and good formulation stability.

Figure 4: Physical Appearance Anti-Acne Gel Formulation

2. pH Evaluation

Table 6: pH of Anti-Acne Gel Formulations

Formulation	pH
F1	7.0
F2	7.0
F3	6.0

The pH of the formulated gels was found to be in the range of 6.0 to 7.0, which is close to the physiological pH of the skin. This indicates that the formulations are non-irritant and suitable for topical application. The neutral to slightly acidic pH helps maintain the natural skin barrier and reduces the risk of irritation or dryness. Among the formulations, F1 and F2 exhibited neutral pH, while F3 showed slightly acidic pH, which may enhance antimicrobial activity but still remains within an acceptable range for skin compatibility.

3.Spreadability

The spreadability of the formulated gels was evaluated to determine their ease of application on the skin. A small quantity of gel was placed between two glass slides, and a standard weight was applied to form a uniform thin film. The time required for the upper slide to move a specified distance under the applied weight was recorded, and spreadability was calculated.

S=M×LTS = \frac {M \times L} {T}S=TM×L

Where:
S = Spreadability (g·cm/sec)
M = Weight applied (g)
L = Length of glass slide (cm)
T = Time taken (sec)

Table 7: Spreadability of Anti-Acne Gel

Formulation	Weight (g)	Length (cm)	Time (sec)	Spreadability (g·cm/sec)
F1	15	7.5	7.23	3.5
F2	15	7.5	10.0	3.0
F3	15	7.5	7.97	3.4

The spreadability values of the formulated gels were found to be in the range of 3.0–3.5 g·cm/sec, indicating good applicability of the formulations on the skin. Among the three formulations, F1 exhibited the highest spreadability (3.5 g·cm/sec), which suggests that it requires less time and effort to spread, making it more suitable for topical application. This improved spreadability can be attributed to its optimal viscosity and balanced composition.

Formulation F2 showed the lowest spreadability (3.0 g·cm/sec), which may be due to its comparatively higher viscosity, resulting in greater resistance to flow. F3 demonstrated moderate spreadability (3.4 g·cm/sec), indicating acceptable performance but slightly lower efficiency compared to F1.

Overall, the results indicate that spreadability is inversely related to viscosity, and formulation F1 provides the best balance between ease of application and consistency. Good spreadability ensures uniform distribution of the gel over the skin surface, which is essential for effective drug delivery and patient compliance.

4.Washability Study

Washability is an important parameter for topical formulations, as it reflects the ease with which the product can be removed from the skin surface after application. The washability of the prepared anti-acne gel formulations was evaluated by applying a small quantity of gel on the skin and observing its removal with water.

Table 8: Washability of Anti-Acne Gel

Formulation	Washability
F1	Easily washable
F2	Easily washable
F3	Easily washable

All the formulated gels (F1, F2, and F3) were found to be easily washable with water, indicating good formulation characteristics. This property suggests that the gel does not leave any sticky or greasy residue on the skin after washing, which enhances user comfort and acceptability.

The ease of washability can be attributed to the hydrophilic nature of the gel base (Carbopol 934) and the proper incorporation of excipients, which allows the formulation to disperse readily in water. Good washability is particularly desirable for anti-acne formulations, as it ensures convenient removal from the skin without causing irritation or clogging of pores.

Overall, all formulations exhibited satisfactory washability, making them suitable for topical application and improving patient compliance.

Viscosity Study

Viscosity is a critical parameter in the evaluation of topical gel formulations, as it influences spreadability, stability, and overall patient acceptability. The viscosity of the prepared anti-acne gel formulations was measured using a Brookfield viscometer under controlled conditions. The measurements were carried out at 25°C using spindle number 64 at a rotational speed of 10 rpm.

Table 9: Viscosity of Anti-Acne Gel

Formulation	Viscosity (cP)
F1	3100 cP
F2	3900 cP
F3	4700 cP

The viscosity of the formulated gels was found to range from 3100 to 4700 cP, indicating that all formulations possess suitable rheological properties for topical application. Among the formulations, F1 exhibited the lowest viscosity (3100 cP), which contributes to better spreadability and ease of application on the skin. In contrast, F3 showed the highest viscosity (4700 cP), indicating a thicker consistency, which may reduce spreadability but can enhance retention time on the skin surface.

F2 demonstrated intermediate viscosity (3900 cP), suggesting a balance between consistency and spreadability. The variation in viscosity among formulations can be attributed to differences in the concentration of the plant extract and its interaction with the Carbopol gel matrix.

The results also indicate an inverse relationship between viscosity and spreadability, where formulations with lower viscosity (F1) showed higher spreadability, while those with higher viscosity (F3) exhibited reduced spreadability. An optimal viscosity is essential to ensure that the gel remains stable, does not flow excessively, and spreads uniformly on the skin.

Overall, formulation F1 was found to be optimal, as it provides a desirable balance between viscosity and spreadability, making it suitable for effective topical delivery.

Parameters Evaluated (Stability Study) :

Table 10: Batch F1 (Room Temperature)

Parameter	**0 Days**	**5 Days**	**10 Days**
Physical appearance	No change	No change	No change
pH	7.0	7.0	7.0
Spreadability (gm.cm/sec)	3.0	2.5	2.3
Homogeneity	Smooth, uniform	Smooth	Smooth
Washability	Easily washable	Easily washable	Easily washable
Consistency	Smooth	Smooth	Smooth

Table 11: Batch F1 (Refrigerated 2–8°C)

Parameters	**0 Days**	**5 Days**	**10 Days**
Physical Appearance	No Change	No Change	No Change
pH	7.0	7.0	7.0
Spreadability (gm.cm/sec)	3.5	2.0	2.5
Homogeneity	Smooth &Uniform	Smooth	Smooth
Washability	Easily Washable	Easily Washable	Easily Washable
Consistency	Smooth	Smooth	Smooth

Table12: Batch F2 (Room Temperature)

parameters	**0 Days**	**5 Days**	**10 Days**
Physical appearance	No change	No change	No change
pH	7.0	6.5	5.2
Spreadability (g.cm/sec)	3.5	2.0	1.5
Homogeneity	Smooth & uniform	Smooth	Slight Change
Washability	Easily washable	Easily washable	Easily washable

Consistency	Smooth	Smooth	Slight Change

Table13: Batch F2 (Refrigerated 2–8°c)

Parameters	**0 Days**	**5 Days**	**10 Days**
Physical appearance	No change	No change	change
pH	7.0	6.5	6.0
Spreadability (g.cm/sec)	3.5	2.0	2.2
Homogeneity	Smooth	Smooth	Moderate
Washability	Easily washable	Easily washable	Easily washable
Consistency	Smooth	Smooth	Smooth

Table14: Batch F3 (Room Temperature)

Parameters	**0 Days**	**5 Days**	**10 Days**
Physical appearance	No change	No change	Slight change
pH	6.0	6.5	5.5
Spreadability (g.cm/sec)	3.4	2.0	2.0
Homogeneity	Smooth, uniform	Smooth	Slight change
Washability	Easily washable	Easily washable	Easily washable
Consistency	Smooth	Smooth	Slight change

Table15: Batch F3 (Refrigerated 2–8°c)

Parameters	**0 Days**	**5 Days**	**10 Days**
Physical appearance	No change	change	Change
pH	6.0	6.0	6.5
Spreadability (g.cm/sec)	3.4	2.6	3.0
Homogeneity	Smooth	Slight change	Moderate
Washability	Easily washable	Easily washable	Easily washable
Consistency	Smooth	Slight change	Moderate change

The short-term stability study indicated that the anti-acne gel formulations exhibited varying degrees of stability under different storage conditions over a period of 10 days. Formulation F1 showed the highest stability, as no significant changes were observed in physical appearance, pH, homogeneity, washability, or consistency under both room temperature and refrigerated conditions. Although a slight decrease in spreadability was noted, the formulation remained within acceptable limits, indicating good stability.

In contrast, Formulation F2 showed noticeable instability, particularly at room temperature, where a gradual decrease in pH and spreadability was observed along with slight changes in homogeneity and consistency by day 10. Even under refrigerated conditions, minor variations were noted, suggesting moderate stability.

Formulation F3 exhibited moderate stability, with slight changes in physical appearance, homogeneity, and consistency under both storage conditions. Variations in pH and spreadability were also observed, indicating that the formulation is less stable compared to F1.

Overall, the study demonstrates that lower storage temperatures enhance formulation stability, and among all formulations, F1 was found to be the most stable and suitable for further development.

Antimicrobial Activity:

Table 16: Antimicrobial Activity of Anti-Acne Gel Against *Cutibacterium acnes* (Zone of Inhibition in mm)

Sr. No	Sample	Zone of Inhibition (mm) P. acne
1	Standard Cream (1 mg/ml)	28
2	Sample F1 (5 mg/ml)	01
3	Sample F1 (10 mg/ml)	02

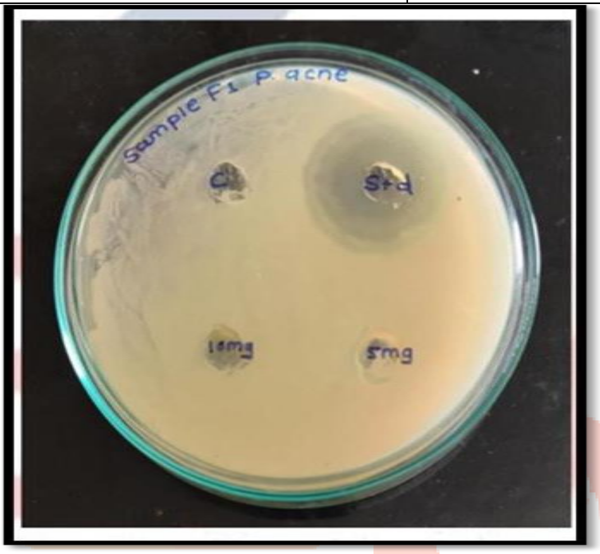

Figure 5: Propionibacterium acne ATCC No-11827

The results indicate that the standard cream exhibited a significantly higher antimicrobial activity with a zone of inhibition of 28 mm, confirming its strong effectiveness against *Cutibacterium acnes*. In comparison, the formulated gel (F1) showed very low antimicrobial activity, with zones of inhibition measuring 1 mm and 2 mm at concentrations of 5 mg/mL and 10 mg/mL, respectively.

Although a slight increase in activity was observed with increasing concentration, the overall antimicrobial effect of the formulation was considerably lower than the standard. This reduced activity may be attributed to factors such as low concentration of active phytoconstituents, poor diffusion of the extract through the gel matrix, or possible interaction between formulation components that may limit the release of active compounds.

Overall, the formulation demonstrated minimal but measurable antibacterial activity, suggesting that further optimization is required to enhance its antimicrobial efficacy.

CONCLUSION

The present study successfully developed an anti-acne gel formulation incorporating *Psidium guajava* leaf extract and evaluated its physicochemical and antimicrobial properties. The formulated gels exhibited satisfactory organoleptic characteristics, appropriate pH, good spreadability, acceptable viscosity, and excellent washability, making them suitable for topical application.

Among the three formulations, F1 was identified as the most optimized formulation due to its balanced viscosity, superior spreadability, and highest stability under different storage conditions. The short-term stability study confirmed that lower temperatures favor formulation stability, with minimal changes observed in F1 over the study period.

However, the antimicrobial study revealed that the formulated gel showed relatively low activity against *Cutibacterium acnes* when compared to the standard drug. This suggests that while the formulation is pharmaceutically acceptable, its therapeutic efficacy can be further improved by optimizing the concentration of active constituents, enhancing drug release, or modifying the formulation strategy.

Overall, the study highlights the potential of *Psidium guajava* as a natural therapeutic agent for acne management and provides a foundation for further research to develop a more effective and clinically viable herbal anti-acne formulation.

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