Research Article ISSN: 2349-2678

Contents lists available at www.ijpba.in
*International Journal of Pharmaceutical and Biological Science Archive*
NLM (National Library of Medicine ID: 101738825)
*Index Copernicus Value 2019: 71.05*
Volume 11 Issue 2; March-April; 2023; Page No. 34-42

# Formulation and Evaluation of Herbal Gel for Acne

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**Article Info:** Received: 05-02-2023 / Revised: 20-02-2023 / Accepted: 05-03-2023
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**Conflict of interest statement:** No conflict of interest

## Abstract

Averrhoea bilimbi is used as a folk medicine for a variety of ailments. Averrhoa bilimbi is a medicinal tree that has been used to cure a variety of medical ailments in the tropical and subtropical climates, particularly in Asia. Averrhoea bilimbi includes the flavonoids luteolin and apigenin, which are antimicrobial and can be used to treat acne. Bilimbi has the potential to be employed as an anti-acne medicine derived from active extracts due to its ability to decrease inflammation and the growth of Staphylococcus aureus germs, which are the reasons producing acne. The development was carried out in this study by turning a chloroform and acetone extract from Averrhoa bilimbi leaves into a gel. The antibacterial activity of acetone and chloroform extracts of Averrhoa bilimbi leaves is tested in vitro using the well diffusion method. The air-dried leaves of A. bilimbi were steeped in acetone and chloroform (1:10; wv-1) for 24 hours before being filtered. To obtain the crude dried extract, both extracts were evaporated to dryness. Anti-acne gel prepared from these extracts with carbopol as a gelling agent has antibacterial activity against Gramme positive (S.aureus) and Gramme negative (E.coli) bacteria. The phytochemical screening of Averrhoea bilimbi leaf extracts reveals the presence of alkaloids, flavonoids, and sterols. The gel formulation was examined for organoleptic properties, homogeneity, pH, grittiness, spreadability, viscosity, and extrudability. Antibacterial activity was tested using the well diffusion method, and bacterial growth inhibition was compared to clindamycin as a positive control. The pH of the acetone extract was found to be 6.400.05, and the pH of the chloroform extract was found to be 6.430.05. None of them caused skin discomfort. Clindamycin had a 36.30+0.37 inhibitory activity against Staphylococcus aureus bacteria.

## Introduction

Acne is the most common skin illness among various skin disorders. Almost everyone has had acne-prone skin, especially as a teen. Although acne is not a hazardous disease, practically all acne sufferers have a disrupted appearance, which often leads to a loss of confidence and interferes with their everyday activities.

Acne vulgaris is characterised clinically by scaly red skin (seborrhea), erythematous papules and pustules, comedones, nodules, deep pustules, and occasionally pimples. Acne's pathogenicity mechanisms included sebum production, follicular hyperkeratinization, bacterial colonisation, and inflammation [1]. P. acnes, S.epidermidis, S.aureus, Klebsiella pneumoniae, Streptococcus, Enterobacter, and other bacteria isolated from acne sufferers have been linked to acne pathogenesis [2]. Staphylococcus aureus is a kind of bacteria.S. aureus, the most prominent member of the skin microbiota, is a pathogen in many skin illnesses, including folliculitis and impetigo, and their co-existence with other microorganisms in acne lesions has been shown. The pathogen creates extracellular matrix and serum binding proteins such as adhesins [surface protein (SasG)] and the fibronectin binding proteins FnBP-A and FnBP-B in order to penetrate the host cell. By linking them to cellular integrins, these factors aid in

their internalisation into host cells. Once a pathogen infiltrates the human epidermis, it begins manufacturing extracellular enzymes such as proteases, lipases (geh1), hyaluronidases, and collagenase, which aid in tissue degradation and hence aid in the pathogen's spread into deeper tissues. Furthermore, they are recognised for producing exfoliative toxins such as enterotoxins A-E, toxic shock syndrome toxin-1, Panton-Valentine leucocidin, leukocidin E-D, S. aureus exotoxin, and cytotoxins such as -, -, - hemolysins during their pathogenic life cycle. The organism produces enzymes such as staphylokinase (sak) and aureolysin: the former binds to defensins, preventing them from acting against the pathogen, while the latter binds and cleaves human cathelicidin LL-37, providing additional protection for the pathogen to establish its pathogenicity in the human system.[2] When bacteria colonise the comedons, the bacteria release inflammatory chemicals. As a result, the comedones morphed into pustules and pimples. After healing, the inflamed acne ruptures and develops nodules, as well as scars. The treatment will be determined by the type of acne, the severity of the acne, the number of lesions, and the anatomic position. Acne can be treated with either topical or systemic medication. Topical treatments include antibiotics, anti-inflammatory medications, and comedolytic drugs. Acne can be efficiently treated with benzoyl peroxide or its combination with clindamycin or erythromycin. Oral antibiotics such as tetracycline and its derivatives were the initial choice for systemic therapy. It is mostly used to treat moderate-to-severe inflammatory acne. However, long-term oral antibiotic medication not only causes bacterial resistance but also increases the risk of upper respiratory tract infection. Because of the presence of bacterial resistance and unforeseen side effects, traditional medicine has the potential to replace the effectiveness of synthetic medications in treating acne vulgaris. [1] Plants have been a source of inspiration for innovative medicinal molecules since ancient times, and plant-derived medicines have made significant contributions to human health and well-being. Many Indian plants are used in medicine for their anti-diabetic and antibacterial properties. Averrhoa bilimbi (Bilimbi) is a folk treatment for a variety of illnesses. It is used to cure fever, mumps, pimples, rectum inflammation, and diabetes, as well as itches, boils, rheumatism, syphilis, bilious colic, whooping cough, hypertension, stomach discomfort, ulcer, and as a cooling drink.[3]

**Classification of Averrhoa bilimbi, L**

| **Kingdom** | Plantae-Plants |
|---|---|
| Subkingdom | Tracheobionta-Vascular plants |
| Superdivision | Spermatophyta-Seed plants |
| Division | Magnoliophyta-Flowering plants |
| Class | Dicotyledonae |
| Subclass | Rosidae |
| Order | Oxalidales |
| Family | Oxalidaceae |
| Genus | Averrhoa |
| Species | bilimbi L. |

The bilimbi, Averrhoa bilimbi, L., (Oxalidaceae), is closely allied to the carambola but quite different in appearance, manner of fruiting, flavor and uses. The only strictly English names are "cucumber tree" and "tree sorrel", bestowed by the British in colonial times. "Bilimbi " is the common name in India and has become widely used.[3]

Other names for Averrhoa obtusangula stokes include Belimbing asam, Belimbing buluh, Belimbing wuluh, Kamias, kalamias, Iba, kolonanas, and Ta-ling-pring.

**Description**

The tree is elegant and long-lived, reaching heights of 16 to 33 feet (5-10 metres), with a small trunk that quickly divides into a variety of

upright branches. The leaves are alternate, imparipirmate, 12 to 24 in (30-60 cm) long, with 11 to 37 alternate or sub opposite leaflets, ovate or oblong, with rounded base and pointed tip; downy; medium-green on the upper surface, pale on the underside; 3/4 to 4 in (2-10 cm) long, 1/2 to11/8in (1.2-1.25 cm) wide. Small, fragrant, 5-petaled flowers, yellowish-green or purplish with dark-purple markings, are borne in small, hairy panicles that emerge directly from the trunk and oldest, thickest branches and some twigs, as are clusters of strange fruits. The bilimbi is ellipsoid, obovoid, or almost cylindrical in shape, 1 1/2 to 4 in (4-10 cm) long, with a thin, star-shaped calyx at the stem end and 5 hair-like floral remains at the apex. When unripe, the fruit is crisp; when mature, it changes from brilliant green to yellowish-green, ivory, or virtually white, and falls to the ground. The skin is glossy, very thin, delicate, and tender on the outside, and the meat is green, jelly-like, juicy, and intensely acidic on the inside. There may be a few (six or seven) flattened, disc-like seeds approximately 1/4 in (6 mm) broad, smooth, and brown.[3]

**Figure 1**: Averrhoa bilimbi

Tannins, saponins, flavonoids, and alkaloids have all been discovered in Bilimbi.A. bilimbi leaves have also been reported to contain potentially toxic chemicals such as flavonoids. Many studies have been conducted to investigate the use of phenolic and flavonoid chemicals as antioxidants and antibacterial agents. Phenolic acids such as benzoic, vanillic, sinapic, and p-hydrouxy benzoic acids, as well as avoboid compounds such as quercetin, rutin, and kaemferol, have been shown to have antioxidant and antibacterial properties. [4] Averrhoa bilimbi has the potential to be employed as an anti-acne medicine based on its action in decreasing inflammation and proliferation of Staphylococcus aureus bacteria, which are the reasons producing acne.[5] Extraction techniques are commonly used to isolate biologically active chemicals from plant matrices.Maceration is a typical extraction process for separating active chemicals from plant matrix. The cell wall and membrane would be destroyed in this procedure, allowing secondary metabolites to escape from the cytoplasm and dissolve in the solvent.An active component derived from plant matrix is a crucial factor in the development of a functional food or therapeutic medicine.[4] The development was carried out by turning a chloroform and acetone extract from Averrhoa bilimbi leaves into a gel, which was then evaluated on the growth of Staphylococcus aureus bacteria.

Gel formulations can be utilised as an anti-acne treatment alternative. The gel dosage form has a long shelf life in the skin and allows for the release of beneficial active ingredients. The gel consistency makes the preparation easier to remove from the skin than ointment or cream. The purpose of this study was to create an anti-acne gel formulation of A.bilimbi using carbopol as a gelling agent because another study found that carbopol provided superior drug release in gel formulations.[1]

## Methods and Materials

### Instruments and chemicals

Chloroform, acetone, carbopol 940, propylparaben, glycerin, triethanolamine,

distilled water, and Muller Hinton agar were employed as compounds. Staphylococcus aureus and E.coli were the microorganisms used. Analytical scales, glassware, a blender, a pH metre, a micropipette, an oven, an autoclave, and an incubator were all used.

**Plant material extraction and collection**

Averrhoa bilimbi leaves were obtained from its native habitat in Thrissur, Kerala, India, in November-December 2021. Fresh A.bilimbi leaves were air dried for 72 hours at room temperature, crushed into minute pieces, and individually soaked for 24 hours in a 1:10 (w/v) ratio of chloroform and acetone.The chloroform and acetone extract was filtered and thoroughly evaporated at room temperature.[6]

**Screening for Phytochemical Implications**

The usual protocols were used to conduct qualitative phytochemical screening of a selected plant's leaf extract.

**Check for Alkaloids**

• Mayer's test: 1 ml of extract was treated with a drop or two of Mayer's test reagent along the edges of the test tube and the formation of white or cream-colored precipitate was seen.
• Wagner's test: 1 ml of extract was treated with Wagner's reagent along the edges of the test tube and the formation of a reddish brown colour precipitate was noticed.
• Hager's test: 1 mL of extract was treated with 1 or 2 mL of Hager's reagent and the production of a noticeable yellow precipitate were noted.

**Tannins Test**

• Ferric chloride test: 0.5 g extract was mixed with 10 ml of distilled water and then filtered. A few drops of 1% ferric chloride solution were added to 2 ml of filtrate and the blue-black, green, or blue-green precipitate was noticed.
• Ferric chloride test: The extract (50 mg) was dissolved in 5 ml of distilled water and treated with a few drops of 5% ferric chloride, and the production of a dark green colour was noticed.
• Lead acetate test: The extract (50 mg) was diluted in 5 mL of distilled water, then 3 mL of 10% lead acetate solution was added and the development of bulky white precipitate was seen.

**Flavonoid Testing**

• NaOH test: 1 mL of the extract was dissolved in water and filtered; 2 mL of 10% aqueous sodium hydroxide was added afterwards to generate a yellow coloration. The presence of flavonoids was shown by a change in hue from yellow to colourless when weak hydrochloric acid was added.
• Lead acetate test: Fifty milligrammes of extract were placed in a test tube, and a few drops of lead acetate solution were added before looking for yellow precipitate.
• Liebermann-Burchard test: The extract (50 mg) was dissolved in 2 ml of acetic anhydride. One or two drops of Conc. H2SO4 were placed along the side of the test tube and any colour change was observed.
• Liebermann-Burchard test: A little amount of extract (50 mg) was dissolved in ethanol. 1 ml of acetic anhydride was added to it, followed by Conc. H2SO4. Terpenoids are present when the colour changes from pink to violet.

**Check for Saponins**

• Foam Test: Distilled water was used to dilute the extract (50 mg) or dry powder to a volume of 20 ml. After aggressively shaking the solution for 15 minutes, the creation of a 2 cm layer thick foam is noticed.

**Analyse for Anthraquinones**

• Borntrager's test: To be tested extract (0.2 g) was mixed with 10 ml of benzene and then filtered. Five millilitres of 10% ammonia solution were added to the filter, agitated, and looked for the appearance of a pink, red, or violet tint.

**Protein Testing**

• Ninhydrin test: Three drops of ninhydrin solution (10 mg ninhydrin in 200 ml acetone) were added to 2 ml of extract and the presence of the typical purple hue was noted.

• Biuret test: One drop of 2% copper sulphate solution was added to two millilitres of extract. 1 ml of 95% ethanol was added to this, followed by an excess of potassium hydroxide pellets, and the creation of a pink ethanolic layer was noticed.

**Search for Quinones**

• H2SO4 test: 1 mL of extract was mixed with 1 mL of Conc. H2SO4 and the development of red colour was noticed.

• HCl test: To 1 mL of extract, 5 mL of HCl was applied and the presence of yellow colour precipitate was noticed.[7]

**Result**

**Table 1:** Phytochemicals present in A.bilimbi leaf extract

| Sl. No | Constituents | Acetone Extract | Chloroform Extract |
|---|---|---|---|
| 1 | Alkaloids | + | + |
| 2 | Flavonoids | + | + |
| 3 | sterols | + | + |
| 4 | Terpenoids | - | + |
| 5 | Anthraquinones | - | - |
| 6 | Phenols | + | - |
| 7 | Saponins | - | + |
| 8 | Tannins | + | + |
| 9 | Proteins | - | - |
| 10 | Quinones | - | + |

"+"presence "-"absence

**Formulation of Gel**

Gels are semisolid preparations for the skin that are easier to apply than lotions and creams. Creams and lotions are rapidly removed from the skin, resulting in poor medication absorption. Applying the gel to the skin is convenient because it is spreadable and washable, eliminating the need to remove it from the skin. Furthermore, regardless of the active agent's water solubility, gel application has the capacity to release active compounds instantly. The A. bilimbi leaf gel has anti-acne characteristics that are pleasant, effective, and simple to use. Carbopol was selected as a gelling agent since it was believed to have more gelling properties than another. Polymer Carbopol is also not absorbed by the body and does not cause inflammation. In the gel formulation, carbopol polymer proven to be a viable carrier for regulated release of active phytoconstituents. Another study indicated that gel formulations created with Carbopol as a gelling agent outperformed gel formulations. As a result, the effective concentration of formulation carbopol for the optimal base gel formulation was identified. Carbopol is a synthetic polymer composed of cross-linked carbomers that form a microgel structure. Because carbopol is anionic in nature, it must be neutralised for microgel construction by adding triethanolamine. Because triethanolamine contains 56 to 86% carboxylic acid, it was introduced as a neutralising agent for acidic carbopoles. Triethanolamine also works as a carbopol stabiliser and developer, preventing carbopol dispersion disruption when the gel becomes hazy due to light exposure. As a preservative, methylparaben is added. This is due to the use of a water medium, which is extremely susceptible to microbial development. Meanwhile, triethanolamine was added to the mixture in order to correct the pH.[1]

**Table 2:** Formula of averrhoa bilimbi leaf gel

| SL.NO | Ingredients | Formula 1 | Formula 2 |
|---|---|---|---|
| 1 | Averrhoa bilimbi leaf extract | 1g | 1g |
| 2 | Carbopol 940 | 1g | 1g |
| 3 | Methyl paraben | 0.2g | 0.2g |
| 4 | Glycerin | 5g | 5g |
| 5 | Triethanolamine | 1g | 1g |
| 6 | Distilled water | 100ml | 100ml |

**Procedure**

**The chemicals required are weighted in accordance with the formula.**

Place the carbopol 940 in a clean, dry mortar. Then dissolve it in hot water and crush it to make a gel base.Crush the methyl paraben and glycerin until homogenous.Then, once the base is uniform, add the extract and triethanolamine.Then, add distilled water and homogenise to produce a gel.The ready-to-use gel compositions are then placed in the container. [5]

**Gel Evaluation**

**1. Physical Appearance**

Colour, texture, and consistency of produced compositions were evaluated physically. Observations were made. [9]

**2. Ph Determination**

The pH metre was calibrated with standard neutral buffer solution (pH 7.01) and acid buffer solution (pH 4.01) until the instruments displayed pH values. The electrode is then rinsed with distilled water and dried with tissue. Distilled water was used to dilute 1g of the gel to be tested. The electrodes are immersed in the solution being tested, and the equipment is able to display the pH of the solution. The pH of the gel must match that of the skin (4.5-6.5). [5]

**3. Homogeneity Evaluation**

The uniformity of the formulations was evaluated visually after the gels were set in a piece of glass or a suitable transparent material. There are no coarse grains and the structure is homogeneous. [5]

**4. Test for Grittiness**

The presence of particles in the formulation was examined microscopically. [8]

**5. Test for Dispersion**

A 0.1g acetone and chloroform extract gel is weighed and placed in the centre of a glass slide, followed by another glass slide placed over the gel mass for 1 minute. The length of the dispersed gel was measured, then 30g of extra weight was added, the gel spread was measured after 1 minute, and the length of the gel spread was recorded. The weight is gradually increased until a consistent length is reached. [5]

**Test for Antibacterial Activity**

All tools used in the antibacterial test were well cleaned and wrapped in paper. Glass (heat-resistant) tools are sterilised in an oven at 160-170 °C for 1-2 hours, and bacterial growth media are sterilised in an autoclave at 121 °C for 15 minutes. The Muller Hinton agars are dissolved in an Erlenmeyer flask with distilled water (38.0 g/1000 ml), then covered securely with a cotton pad coated in paper and tied with a rope. Then, over a water bath, homogenise it until it boils. Then sterilise it in an autoclave for 15 minutes at 121 degrees Celsius.

Prepare a petri dish that has been sterilised.Allow 60 ml of Mueller Hinton agar media to solidify. The cells were then injected with 0.1 ml of bacterial solution. Pits were also created using a metal holder. The wells are then filled with gel preparation in the amount of 0.05 g. The resistivity zone (clear zone) that forms is then measured after it has been incubated in an incubator for 24 hours at a temperature of 35-37 °C. [5]

**Results and Discussion**

**1. Physical Appearance**

The resulting gel had a semi-solid form, pale yellow to greenish in colour, and the ensuing odour was the distinctive aroma of the extract from each preparation, according to the organoleptic test results of Averrhoa bilimbi L. extract gel. The organoleptic test results are shown in the table.

**Table 3:** Physical appearance

| Formulas | Consistence | Color | Odour |
|---|---|---|---|
| F1 | semisolid | Yellow transparent | The distinctive smell of extract |
| F2 | semisolid | Pale green | The distinctive smell of extract |

F1- *A.bilimbi* leaf chloroform extract
F2-*A.bilimbi* leaf acetone extract

**2. Ph Determination**

The pH of acetone leaf extract gel formulations was around 6.400.05, whereas the pH of chloroform leaf extract gel formulations was around 6.430.05.


**3. Homogeneity Test**

The Chloroform Leaf Extract and acetone leaf extract shows good homogeneity.

**Table 4:** Homogeneity test

| **Formula** | **Homogeneity** |
|---|---|
| F1 | Homogeneous |
| F2 | Homogeneous |

**4. Test for Grittiness**

Microscopically, no particles were discovered in the formulations.

**5. Test for Dispersion**

Both acetone and chloroform extract gels were used for the dispersion test. The length of dispersion in chloroform extract gel is 1.8 cm, while in acetone extract gel it is 1.3 cm. The test is performed to determine the consistency of the preparation as well as to evaluate how the preparation disperses when applied to the skin. As a result, it is believed that the active chemicals will be distributed equally across the skin's surface. The results of the tests reveal that the gel is consistent and produces good surface dispersion.

**Test for Antibacterial Activity**

Table 5 shows the results of the antibacterial activity test of Averrhoa bilimbi ethyl acetate extract gel against Staphylococcus aureus. The MIC values from this study were not computed. This investigation was limited to the ratio of the gel's inhibition zone diameter to bacterial growth on nutrient agar media.

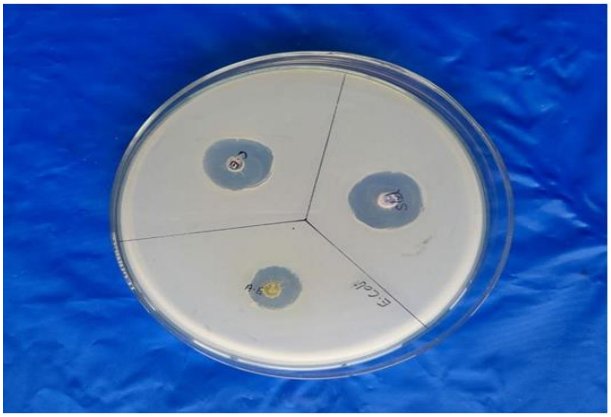
The positive control was a gel containing clindamycin (Mediklin®), and the negative control was a gel base devoid of active ingredients. The test's inhibition value revealed that the positive control had a very strong ability compared to the other groups, while the negative control had no inhibitory power against bacterial growth. When compared to the negative control, the formula containing the test sample demonstrated considerable inhibition (p0.05). Based on the data, it is possible to assume that the higher the concentration of extract in a specific formulation, the stronger the antibacterial activity of the gel. This is consistent with Pelzer and Chan's idea that the higher the concentration of antimicrobial chemicals evaluated, the greater the antibacterial activity of these compounds.

The clear zone surrounding the well is caused by the active material content of Averrhoa bilimbi fruit ethyl acetate extract gel, which contains antibacterial flavonoids, alkaloids, saponins, terpenoids, and tannins. Through hydrogen bonding, flavonoid compounds can form complexes with bacterial cell proteins. The structure of the cell wall and bacterial cytoplasmic membrane, which contain protein, becomes unstable because the protein structure of bacterial cells is damaged due to hydrogen bonds with flavonoids, causing the bacterial cell protein to lose its biological activity, resulting in bacterial cell death. Alkaloids contain antibacterial and inhibitory mechanisms that work by interfering with the components of the

peptidoglycan composition of bacterial cells, causing the wall layer to fail and causing bacterial death.

**Table 5:** Antimicrobial activity of different plant extracts and formulated gels

| Sl.No | Formulation | Name of organism | Time of incubation | Zone of inhibition in (mm) | Inhibition zone with clindamycin |
|---|---|---|---|---|---|
| 1 | Chloroform extract of A. bilimbi | Staphylococcus aureus | 24 hrs | 9 mm | 11 mm |
| | | E.coli | 24 hrs | 9 mm | 11 mm |
| 2 | Acetone extract of A.bilimbi | Staphylococcus aureus | 24 hrs | 8 mm | 11 mm |
| | | E.coli | 24 hrs | 6 mm | 11 mm |



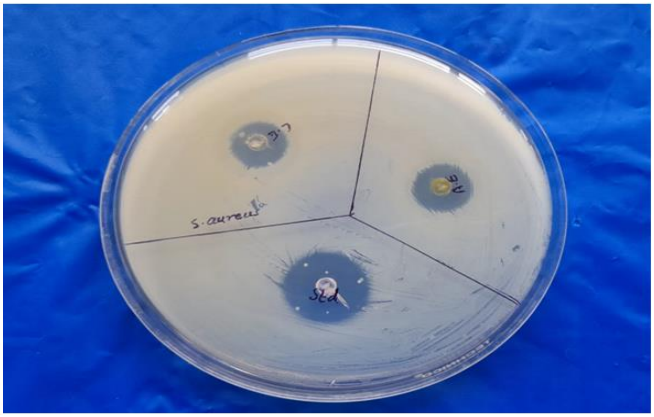


**Figure 5:** Antibacterial activity against E.coli**, Figure 6:** Antibacterial activity against Stapylococcus aureus

## Conclusion

Plants are the primary source of bio-active chemicals, which are used in a variety of biological functions in humans and animals. A. bilimbi is a significant medicinal plant used in traditional medicine to treat a variety of diseases as well as to maintain excellent health and well-being. Extensive pharmacological research has established the scientific basis for the medicinal uses of A. bilimia's leaves and fruits in the treatment of a variety of illnesses and microbial infections over the years. Its antibacterial action inhibits the growth of acne-causing germs. Gel with active extract of Averrhoa bilimbi has good formula qualities and meets all formula assessment requirements. The gel has no skin irritation symptoms and meets the basic standards (Organoleptic, homogeneity, and pH). This gel has strong antibacterial activity, thus it can suppress the growth of the Staphylococcus aureus germs, which are commonly seen in wounds and acne. Chloroform extract gel was shown to have more antibacterial activity than acetone extract gel. According to the findings, Averrhoa bilimbi leaf gel can be utilised as a natural medicine to treat acne, but more research and testing is required. To achieve the best results in patients, the active ingredients must be separated and refined and more clinical trials must be done.

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