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DEVELOPMENT AND EVALUATION OF ANTIBACTERIAL HERBAL LOTION USING *ANNONA SQUAMOSA* LINN. LEAVES AQUEOUS EXTRACT

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Keywords:

Anti-bacterial, Phytochemical screening, *Annona squamosa* Linn, Leave extract, Herbal lotion

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ABSTRACT: The growing demand for natural excipients has catalysed a shift from synthetic sources toward botanical antioxidants and antimicrobial agents. This study evaluates the antioxidant and antimicrobial effectiveness of aqueous extracts from *Annona squamosa* Linn. leaves typically considered agro-waste. The aqueous foliar extract underwent comprehensive phytochemical screening, quantification of total phenolic and alkaloid content, and *in-vitro* antibacterial assessment using the agar well diffusion method. Phytochemical analysis revealed the presence of glycosides, saponins, tannins, flavonoids, and phenols, correlating with notable antioxidant activity. Quantitative assays demonstrated substantial phenolic and alkaloid concentrations, reinforcing the extract's bioactive potential. Antimicrobial testing against key bacterial strains *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* highlighted pronounced inhibitory effects, confirming its broad-spectrum antibacterial efficacy. Building on these findings, an herbal lotion was formulated incorporating the extract alongside carefully selected excipients to enhance dermal delivery and stability. The formulation was subjected to rigorous physicochemical evaluation: pH, viscosity, and spread ability measurements confirmed its compatibility with skin application. Further stability studies under varied environmental conditions demonstrated the lotion's resilience and shelf-life potential. *In-vitro* antibacterial assays using prevalent cutaneous pathogens verified the formulation's efficacy in a cosmetic vehicle. Additionally, dermal irritation testing on suitable models confirmed its safety and non-sensitizing nature for topical use. In summary, this work establishes *Annona squamosa* leaf extract as a potent natural antioxidant and antimicrobial agent, successfully incorporated into a stable, safe, and efficacious topical lotion. These findings support the valorization of plant-based waste materials and advance the development of sustainable, bioactive skincare products.

INTRODUCTION: Antimicrobial resistance (AMR) arises when microbes such as bacteria, fungi, viruses, or parasites evolve to withstand medications previously effective against them, complicating treatment and control of infections.

Recognized by the WHO as a critical threat to global health, AMR is a central concern in efforts outlined in the WHO's Global AMR Research Agenda, which emphasizes the urgent need for novel antimicrobial agents.

Aligning with this global imperative, the objective of this study was to develop a powerful yet safe antibacterial agent derived from *Annona squamosa* Linn. Native to the West Indies, this plant is now widely civilized in India, valued both for its edible fruits and its medicinal uses^{1, 2, 3, 4}. The Ethnobotanical record reported the evoke obtained

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from various parts of the *Annona squamosa*, have been employ and use in conventional pharmacological applications in different countries to cure a variety of diseases, such as insecticidal, an anti-tumour agent, anti-diabetic, antioxidant, anti-lipidemic and anti-inflammatory agent, epilepsy, diarrhoea, heart issues, parasitic infections, constipation, bleeding, fever, and ulcers. Various parts of *A. squamosa* have been utilized in remedying different health issues due to the presence of multiple bioactive substances ^{5, 6}. Pruning of *Annona squamosa* generates large amounts of leaves, posing disposal issues for farmers. However, these leaves hold great value due to their rich bioactive content such as glycosides, flavonoids, alkaloids, and phenols which contribute to antioxidant, antimicrobial, antidiabetic, anticancer, and hepatoprotective properties ^{7, 8, 9, 10}.

In addition, crushed leaves were inhaled to alleviate hysteria and fainting episodes, and applied topically to treat ulcers and wounds. For dysentery, a decoction prepared from the leaves was traditionally consumed ¹¹. The increasing demand for OTC products for daily problems attracts customers. While there are mostly synthetic options are available the market is lacking in the other biodegradable and natural options. This gives us the idea of formulating a natural plant-based option. The increasing consumer interest in natural

and plant-derived skincare solutions has resulted in a significant rise in the appeal of herbal preparations in recent times. Among these, herbal lotions are especially favoured, as they derive from medicinal plant extracts recognized for their beneficial effects on the skin, offering hydration, relaxation, and therapeutic properties. This research is focused on creating and testing a herbal lotion made with plant extracts known for their antibacterial, anti-inflammatory, and moisturizing benefits. The process involves carefully selecting the right ingredients, fine-tuning the method for blending them together, and then evaluating the lotion’s physical properties and its effectiveness in preventing microbial growth. Throughout the development, we aim to ensure that the lotion not only meets skin care needs but also provides natural, gentle care for the skin.

We will also assess how well the lotion performs in real-world conditions, ensuring its safety and effectiveness. To ensure the final product’s consistency, stability, and efficacy, production and analysis are carried out in batches. Here in this research, we are formulating anti-microbial lotion based on the *Annona squamosa* leaf extract for use in basic skin conditions. The detailed characteristics of *Annona squamosa* are summarized in **Table 1**. The plant thrives in tropical conditions, with full sun and slightly acidic soil being ideal.

TABLE 1: PLANT PROFILE ANNONA SQUAMOSA LINN

Category	Details
Synonyms	Sugar apple, Sitaphal, Custard apple
Biological Source	<i>Annona squamosa</i> Linn, Family: Annonaceae
Habitat	Native to the West Indies and South America; cultivated in tropical regions like India and Thailand ¹²
Taxonomy	Kingdom: Plantae, Order: Magnoliales, Family: Annonaceae, Species: <i>Annona squamosa</i> L ¹³
Botanical	Tree: ~6m tall, leaves oblong-lanceolate, fruit globular, yellowish-green when ripe, seeds brownish-black ¹²
Description	
Ecology	Prefers hot, dry climates (30°C), optimal soil pH 6.0-6.5, pollination enhanced at 25°C and 70% humidity ¹⁴

MATERIALS AND METHODS:



FIG. 1: FRESH LEAVE



FIG. 2: DRIED LEAVES

Collection of Plant Material: Mature leaves of *Annona squamosa* Linn. were collected from plants in Nashik on Monday, March 10, 2025. They were thoroughly washed under running tap water to remove dust, then shade-dried at ambient temperature until completely dry. As depicted in **Fig. 1**, the fresh leaves exhibited their natural green appearance before drying. After complete desiccation, the leaves were crushed manually into a coarse powder. The resulting dried leaves powder **Fig. 2** was transferred into a clean, labeled, air-tight container and stored at room temperature for further analysis.

Method for Extraction of *Annona squamosa* Aqueous Extract: Fresh *Annona squamosa* leaves

(50 gm) were carefully weighed and added in 1200 ml of Chloroform water (2.5ml of chloroform in 1200ml water) for 7 days to facilitate extraction of bioactive compounds. The resulting mixture was then filtered with the help of muslin cloth to remove plant residues, ensuring a clear liquid extract. The obtained filtrate was transferred to a China dish and subjected to gentle heating over a water bath to concentrate the extract. Upon evaporation of the liquid, the remaining substance was allowed to cool, after which the condensed material was carefully collected. The final extract, characterized by a dark brown appearance shown in **Fig. 3** and **Fig. 4** was stored at 2–8°C in a refrigerator for further experimental analysis¹⁵.



FIG. 3: POWDER EXTRACT



FIG. 4: POWDER EXTRACT

Pre-formulation: Pre-formulating study was performed of *Annona squamosa* extract powder.

TABLE 2: PRE-FORMULATION RESULTS

Sr. no.	Parameter	Observation
1	Bulk density	0.666 g/ml
2	Tap density	0.9090 g/ml
3	Angle of repose	35.53°
4	Hausner ratio	1.364g/ml

Preliminary Phytochemical Screening: The extracts were analysed using standard phytochemical screening techniques to determine their bioactive components. This evaluation aimed to identify the existence of glycosides, alkaloids, amino acids, flavonoids, carbohydrates, saponins, phenols, steroids, and tannins, providing insight into their potential therapeutic applications¹⁶.

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Quantitative Phytochemical Analysis:

Total Phenolic Content (TPC): The TPC in the leaf extract was quantified using the widely employed Folin–Ciocalteu colorimetric assay, with gallic acid serving as the calibration standard. Serial dilutions of gallic acid (0.01–0.1 mg/ml) were made ready in methanol to create a standard curve. For each assay, 0.5 ml of either the extract or a gallic acid standard was combined with 2 ml of Folin–Ciocalteu reagent (pre-diluted 1:10 in deionized water), followed by 4 ml of saturated sodium carbonate solution (7.5% w/v). The mixture was covered and incubated at ambient temperature for 30 minutes with intermittent shaking. Absorbance measurements were performed at 765 nm, using methanol as the blank, and each

sample was tested in triplicate. Phenolic concentrations were indicating as Gallic acid equivalents (GAE) per gram of dry extract, based on the generated calibration curve. The assay hinges on the reduction of phosphomolybdate-phosphotungstate complexes by phenolic compounds under alkaline conditions, forming a blue molybdenum–tungsten complex that is quantifiable at 765 nm^{18, 19, 20}.

Total Alkaloid Content (TAC): The TAC was estimated using a UV spectrophotometric approach involving bromocresol green (BCG) complexation. Plant extracts at a concentration of 1 mg/ml were dissolved in 2 N hydrochloric acid and filtered to remove particulates. The pH of the phosphate buffer was adjusted to neutrality using 0.1 N sodium hydroxide. From this, 1 ml of the plant extract was transferred into a separating funnel, followed by the addition of 5 ml of BCG reagent and 5 mL of the prepared phosphate buffer. The mixture was vigorously shaken to facilitate complex formation, and the resulting alkaloid-BCG complex was extracted using chloroform. The chloroform layer was collected in a 10 ml volumetric flask and diluted to volume with chloroform. Absorbance was recorded at 470 nm. All measurements were performed in triplicate to ensure accuracy²⁶.

Standard Curve Preparation Using Atropine: Standard solutions of atropine (0.5 to 2.5 ml) were transferred into individual separating funnels. To each, 5 ml of phosphate buffer (pH 4.7) and 5 mL of BCG solution were added. The mixtures were extracted with 4 ml of chloroform and transferred into 10 ml volumetric flasks, with final volume adjusted using chloroform. Absorbance of these solutions was measured at 470 nm against a blank, prepared identically but without atropine. The standard calibration curve obtained was used to calculate the alkaloid concentration in the plant samples^{17, 21, 22}.

Antimicrobial Testing: The antibacterial activity of both herbal and synthetic lotions was evaluated using the disc diffusion method. Bacterial strains, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus*, and *Escherichia coli*, were inoculated onto nutrient agar plates and incubated at 37°C for 24 hours to allow growth. After incubation, filter paper discs infused with each lotion formulation were placed on the plates to assess inhibition zones, indicating antibacterial effectiveness. After another 24-hour incubation, the zone of inhibition was measured indicating how well the formulations prevented bacterial growth. A larger zone meant greater antibacterial effectiveness, helping assess the herbal lotion’s potential as a natural antimicrobial solution^{23, 25}.

Formulation of Herbal Lotion Using *Annona squamosa* Aqueous Extract:

Procedure: To prepare the herbal lotion base, begin by weighing stearic acid, cetyl alcohol, and lanolin accurately, then gently heat them in a water bath at around 75 °C until fully melted to form the oil phase (Part A). In a separate beaker, combine methyl paraben, triethanolamine, glycerol, and rose water, heating this aqueous phase (Part B) to approximately 75 °C. Once both phases reach the same temperature, gradually add the oil phase into the aqueous phase with continuous stirring using either a glass rod or mechanical stirrer until a smooth, uniform emulsion forms. Next, incorporate the *Annona squamosa* leaf extract in geometric progression to prevent lumps and ensure even distribution. Adjust the lotion’s consistency by adding distilled water, then after allowing the mixture to cool slightly gently stir in lemon oil for fragrance the ingredients used in lotion mention in **Table 3**. Finally, transfer the finished herbal lotion into a clean, dry, tightly sealed container, label it with the formulation details, and store it in a cool place for future use or evaluation.

TABLE 3: LOTION FORMULATION TABLE

Sr. no.	Ingredients	F1	F2	F3	F4	Role in Formulation
1.	Stearic Acid	1.0 gm	1.5 gm	1.5 gm	2.0 gm	Emulsifiers Thickeners
2.	Cetyl Alcohol	0.20 gm	0.25 gm	0.10 gm	0.30 gm	Moisturizer
3.	Lanolin	0.30 gm	0.40 gm	0.50 gm	0.60 gm	Emollient
4.	Methyl Paraben	0.05 gm	0.05 gm	0.05 gm	0.05 gm	Preservative
5.	Glycerol	7.0 ml	6.0 ml	4.0 ml	3.0 ml	Humectant&softener
6.	Triethanolamine	0.30 ml	0.30 ml	0.35 ml	0.40 ml	Emulsifying agent
7.	Rose Water	2.0 ml	2.0 ml	1.5 ml	1.5 ml	Skin freshener

8.	Herbal Extract	2.0 ml	2.0 ml	2.0 ml	2.0 ml	Herbal active agent
9.	Lemon Oil	1.0 ml	1.0 ml	1.0 ml	1.0 ml	Fragrance
10.	D. W.up to	30 ml	30 ml	30 ml	30 ml	Solvent/base

The formulation underwent a streamlined evaluation of key quality attributes: organoleptic characteristics (color, odor, texture), pH (measured via calibrated digital meter), washability, irritancy (24-hour skin patch test on a 2 cm² dorsal hand area), homogeneity, spreadability (500 mg between glass slides under specific weights), viscosity (Brookfield viscometer at 20 rpm, 25 °C, triplicate), and a three-month stability assessment at room temperature, which showed consistent pH, viscosity, and spread results indicating a stable, skin-compatible topical product ²⁴.

RESULT AND DISCUSSION: The *Annona squamosa* leaf extract powder demonstrated good flowability and compressibility, making it suitable for formulation. With a bulk density of 0.666 g/ml and tap density of 0.909 g/ml, the powder is well-structured for even blending. The angle of repose (35.53°) suggests smooth handling, while the Hausner ratio (1.364) indicates moderate cohesiveness, ensuring uniform dispersion in lotions the results shown in **Table 4**. These characteristics confirm its potential for stable and effective herbal skincare formulations.

TABLE 4: PRE-FORMULATION STUDY OF POWDER EXTRACT

Sr. no.	Parameter	Observation
1	Bulk density	0.666 g/ml
2	Tap density	0.9090 g/ml
3	Angle of repose	35.53°
4	Hausner ratio	1.364g/ml

The *Annona squamosa* leaf extract powder demonstrated good flowability and compressibility, making it suitable for formulation.

TABLE 5: PRELIMINARY PHYTOCHEMICAL SCREENING

Sr. no.	Phytoconstituent	Observation
1	Steroids	++
2	Phenolic compounds	++
3	Flavonoids	++
4	lycosides	++
5	G	++
6	Saponins	++
7	Alkaloids	++
8	Proteins	++
9	Amino acids	++
10	Tannins	++

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Above **Table 5** of Preliminary phytochemical screening study of the *Annona squamosa* Linn extract shows the presences of Phenolic compound, Flavonoid, Glycosides, Saponins, Triterpenoids, Alkaloid, Tannins, Proteins, Amino acid and Steroid.

Quantitative Phytochemical Analysis:

Alkaloid Content: **Fig. 5** indicates The *Annona squamosa* leaf extract contains 0.149 mg/g of alkaloids, which contribute to its antimicrobial, antioxidant, and anti-inflammatory properties. These compounds help combat infections, support wound healing, and protect the skin, making them valuable for herbal skincare formulations. Extracted using ethanol maceration and quantified via UV-visible spectrophotometry at 470 nm, alkaloid content typically falls within the range of 0.1–0.5 mg/g, depending on extraction conditions.

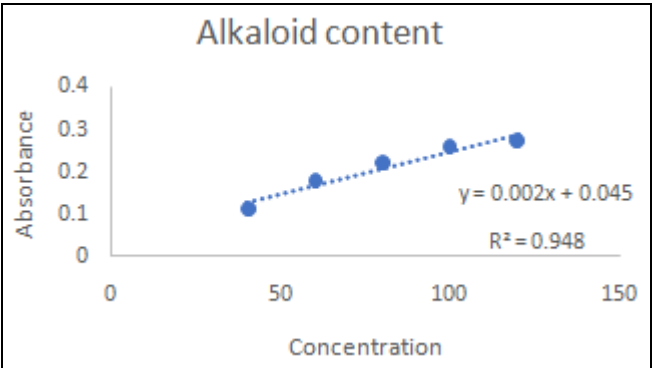


FIG. 5: TOTAL ALKALOID CONTENT

Total Phenolic Content: The *Annona squamosa* leaf extract has a TPC **Fig. 6** was found to be 1.236 -1.25 mg GAE/g, indicating moderate antioxidant potential. Extracted through aqueous maceration and quantified via spectrophotometry at 760 nm, these compounds support antimicrobial and anti-

aging skincare benefits. TPC in such extracts typically ranges between 1.0–2.5 mg GAE/g, depending on extraction conditions. Optimizing methods could enhance its potency for herbal formulations. In the **Table 6** the TPC and TAC results were summarised.

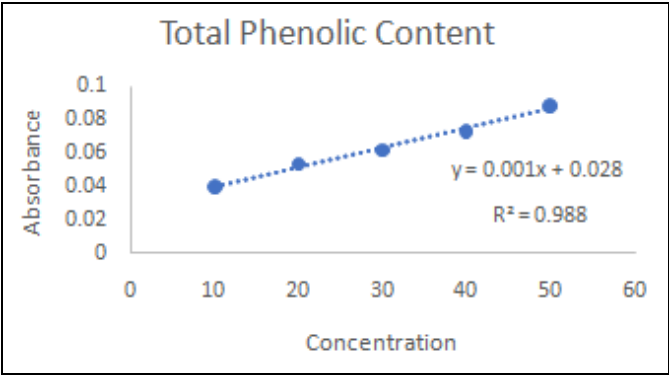


FIG. 6: TOTAL PHENOLIC CONTENT

TABLE 6: TOTAL PHENOL AND ALKALOID CONTENTS OF ANNONA SQUAMOSA LINN. LEAVES EXTRACT

Name of Plant	Family	Part Used	Extract	Total Phenolic Content (GAE) (Mg/G)	Total Alkaloid Content (AE) (Mg/G)
Annona squamosa	Annonaceae	Leaves	Aqueous	1.236 ± 4.714	0.149 ± 4.784

Data are reported as Mean±SD (n=3). GAE: Gallic acid equivalent, AE: Atropine equivalent.

TABLE 7: RESULTS OF ZONE OF INHIBITION USING ANNONA SQUAMOSA EXTRACT IN CM.

Pathogen	50mg (F1)	100mg (F2)	150mg (F3)	200mg (F4)	Std
Escherichia coli (ATCC 25922)	0.5	0.7	0.8	1.0	1.5
Bacillus (NCIM 2250)	0.4	0.5	0.7	0.9	1.6
Pseudomonas aeruginosa (ATCC 27853)	0.4	0.5	0.4	0.6	1.5
Staphylococcus aureus (NCIM 2079)	0.6	0.7	0.7	1.0	1.7



FIG. 7: BACILLUS

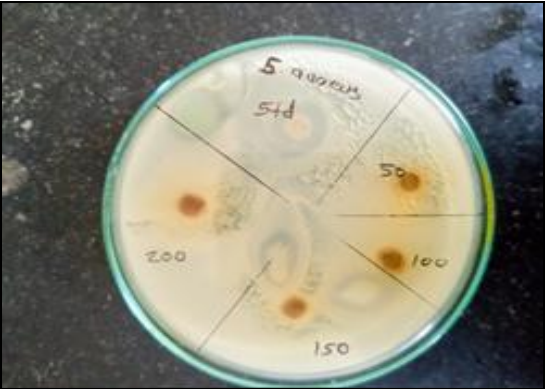


FIG. 8: STAPHYLOCOCCUS AUREUS

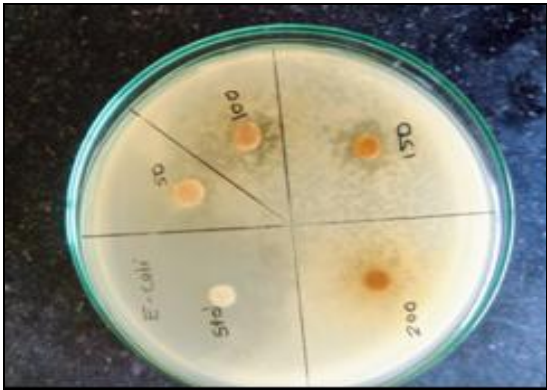


FIG. 9: ESCHERICHIA COLI



FIG. 10: PSEUDOMONAS AERUGINOSA

The *Annona squamosa* leaf extract demonstrated antibacterial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The extract inhibited the growth of these bacteria, indicating its potential as an antimicrobial agent. The zone of inhibition varied depending on the concentration of the

extract, as detailed in **Table 7**. Results of Zone of Inhibition Using *Annona squamosa* Extract in cm. Representative images of the antibacterial activity are shown in **Fig. 7** *Bacillus subtilis*, **Fig. 8** *Staphylococcus aureus*, **Fig. 9** *Escherichia coli*, and **Fig. 10** *Pseudomonas aeruginosa*.

TABLE 8: EVALUATION PARAMETER

Evaluation parameter	F1	F2	F3	F4
Colour	Brownish	Brownish	Brownish	Brownish
Odour	Lemon	Lemon	Lemon	Lemon
Dilution	o/w	o/w	o/w	o/w
Appearance	Smooth	Smooth	Smooth	Smooth
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Grittiness	non gritty	non gritty	non gritty	non gritty
Skin irritation test	No irritation	No irritation	No irritation	No irritation
PH	5.4	5.8	6.2	6.4
Spreadability	7.0cm	7.2cm	7.5cm	7.8cm
Viscosity	24156 cps	21342 cps	23543 cps	22736 cps
Stability (37°C)	Stable	Stable	Stable	Stable
Type of smear	non greasy	non greasy	non greasy	non greasy
Emolliency	no residue left	no residue left	no residue left	no residue left

The four formulations (F1-F4) showed similar characteristics, including brownish colour, lemon odour, smooth texture, and no skin irritation. They had a slightly acidic to neutral pH (5.4-6.4) and were stable at 37°C. The formulations were non-greasy and had varying viscosities and spread

abilities, with F4 showing the highest spread ability, stability and antimicrobial activity. These parameters are detailed in **Table 8**. Evaluation Parameters and the **Fig. 11** is shown the formulation and **Fig. 12** shown the label of the formulation.



FIG. 11: FORMULATION



FIG. 12: LABEL

CONCLUSION: The study successfully demonstrates that herbal lotion formulated with *Annona squamosa* leaf extract can be an effective antimicrobial solution. Traditionally recognized for its bioactive properties, the extract contains alkaloids, flavonoids, phytosterols, saponins, phenols, and glycosides, all of which contribute to its therapeutic benefits. The lotion was developed using a water-in-oil emulsion method, ensuring

optimal absorption and effectiveness against skin infections caused by bacteria. With its smooth application and minimal side effects, this herbal formulation offers a gentle yet powerful alternative to synthetic products. Given that *Annona squamosa* is widely available and cost-effective, its use in skincare formulations aligns with sustainable and natural approaches in cosmetics. The lotion underwent chemical and physical testing,

confirming its stability, antimicrobial activity, and skin-protective properties. Stability studies further validated its long-term effectiveness, reinforcing its potential as a safe, natural anti-bacterial skincare product.

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CONFLICT OF INTEREST: There is no conflict of interest to declare.

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