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RESEARCH

Development and assessment of a polyherbal topical cream comprising *Lantana camara*, *Piper betle*, and *Ocimum sanctum* for antibacterial activity

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The diminishing effectiveness of conventional antibiotics has led to increased interest in herbal alternatives for managing bacterial infections. Plant-based formulations are gaining popularity in dermatology owing to their active constituents, minimal side effects, and broad-spectrum antimicrobial properties. This investigation focused on the formulation and evaluation of a polyherbal topical cream using ethanol extracts of *Lantana camara*, *Piper betle*, and *Ocimum sanctum* (commonly known as Tulsi). The selected plant leaves were shade-dried and extracted via ethanol using a heating mantle method. The obtained extracts were then integrated into a cream base comprising stearic acid, beeswax, glycerine, propylene glycol, and necessary preservatives. The formulated cream underwent various physicochemical analyses such as pH evaluation, spreadability, stability testing, and antibacterial assessment using the agar well diffusion technique. The B3 and B5 batches showed significant inhibition zones of 14 mm against *Staphylococcus aureus* and 20 mm against *Escherichia coli*, respectively. The antibacterial potential was tested against the Gram-positive bacterium *Staphylococcus aureus* and the Gram-negative bacterium *Escherichia coli*. The novelty of this work lies in combining three well-known herbal extracts in a synergistic cream formulation with confirmed antibacterial effects and no observed skin irritation.

Keywords: antibacterial activity, *Lantana camara*, *Ocimum sanctum*, *Piper betle*, polyherbal cream, skin infection treatment

Introduction

Background

Skin infections caused by bacteria and fungi are widespread, affecting millions of people worldwide (1). Synthetic antibiotics and antifungal agents, while effective, are associated with side effects such as skin irritation and antibiotic resistance (2). As a result, herbal medicine has gained attention as a natural, effective, and safer alternative. Herbal medicine, also known as phytotherapy, is one of the oldest kinds of medical treatment that uses plant-derived substances to prevent and treat disease (3). Plants have been used for centuries in traditional medical systems such as Ayurveda, Traditional Chinese Medicine, and Unani because of their antibacterial, anti-inflammatory, antioxidant, and wound-healing properties.

Advantages of the herbal drug system

- Reduced possibility of adverse reaction (1).
- Wide availability (3).
- Works well for chronic ailments (1).
- Low cost-effectiveness (3).
- Herbal medicine efficiently promotes the body's natural detoxification process (1).

Disadvantages of the herbal drug system

- Dosing in bulk (1).
- Poor stability in acidic pH conditions, including hepatic metabolism (3).
- Large patch size limits homogeneous percutaneous absorption (1).
- A significant volume of raw materials is required to reuse the drug (3).

The growing concern over antibiotic-resistant bacteria has fuelled exploration into herbal extracts as implicit natural antibacterials. Plants like *Lantana camara*, *Piper betle*, and *Ocimum sanctum* contain flavonoids, tannins, alkaloids, essential oils, and terpenoids, which exhibit strong antibacterial activity. Their combination in a polyherbal cream enhances their remedial efficacy, making them suitable candidates for topical treatment of microbial skin infections.

This study focuses on the formulation and evaluation of a polyherbal cream containing excerpts of *Lantana camara*, *Piper betle*, and *Ocimum sanctum* to assess its physicochemical parcels and antibacterial eventuality. Polyherbal formulations combine multiple factory excerpts to enhance remedial efficacy through synergistic relations. In this study, a polyherbal cream was formulated using three medicinal plants known for their antibacterial exertion.

1. ***Lantana camara* (Ghaneri)**—Contains flavonoids, terpenoids, and essential canvases that parade antibacterial and antifungal exertion.
2. ***Piper betle* (Paan)**—Rich in phenols, alkaloids, and flavonoids, which give strong antibacterial parcels.
3. ***Ocimum sanctum* (Tulsi)**—Possesses antibacterial, antioxidant, and anti-inflammatory goods.

Ideal

The ideal of this study was to develop a stable polyherbal cream using excerpts from *Lantana camara, Piper betle*, and *Ocimum sanctum* and to estimate its physicochemical parcels and antibacterial exertion against bacterial strains.

Physiology of normal skin

The skin accounts for over 15% of an adult's total body weight, making it the largest organ in the body (4). It serves a variety of critical tasks, including shielding the body from external physical threats that may be chemical or natural, as well as managing temperature and preventing excessive water loss. A continuous layer of skin is formed by the mucous membranes covering the face. The skin and its constituent parts comprise the integumentary system. The epidermis, dermis, and subcutaneous towel are the three layers of the skin. The farthest subcaste, the epidermis, is composed of a special constellation of keratinocytes that make keratin, a long, thread-like protein.

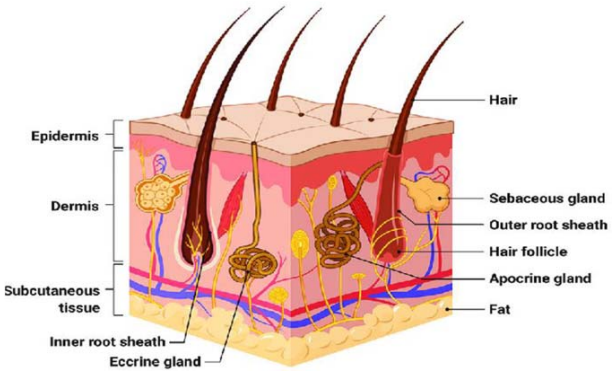


FIGURE 1 | Structure of skin (4).

Collagen is a fibrillar structural protein that makes up the majority of the dermis, or middle subcaste. The panniculus, or subcutaneous towel, on which the dermis rests, is composed of tiny lobes of fat cells called lipocytes. Depending on where the species is disassembled, these layers' densities vary significantly. With a thickness of approximately 1.5 mm, the thickest epidermal subcaste is found on the soles and triumphs of the bases, while the smallest subcaste is found on the eyelid, measuring less than 0.1 mm.

The dermis on the opposite side is 30–40 times thicker than the epidermis around it.

The skin is composed of three layers.

- Epidermis: 50–100 μm.
- Dermis: 1–2 mm.
- Hypodermis: 1–2 mm (4).

The overall architecture of the skin is shown in **Figure 1**.

Percutaneous immersion is hampered by the stratum corneum, the epidermis's most distant subcaste. The stratum corneum works as a permeability barrier, preventing water loss while also protecting against germs and abrasive action.

Corneocytes are flat, polyhedral-shaped cells that are 2–3 μm thick and non-nucleated. They make up the 10–20 μm stratum corneum. Corneocytes are made up mostly of undoable whisking keratin, which is encased in a cell envelope stabilized by cross-linked proteins and lipids that are covalently connected. Corneodesmosomes, also known as membrane connectors, unite corneocytes and help keep the stratum corneum cohesive.

Lipids primarily obtained from lamellar body exocytosis during keratinocyte terminal isolation fill the intercellular space between corneocytes. The healthy functioning of the skin's barrier depends on these lipids. Ten to twenty layers of cells make up the epidermis. Melanocytes, which give skin its color, and Langerhans' cells, which contribute to antigen donation and immunological responses, are also found in this pluristratified epithelium. The dermal vascular network provides nutrition to the epidermis, just like it does to any other epithelium.

Robust stratum corneum regeneration is regulated by complex, nonsupervisory cellular separation processes. Skin hedge dislocations elicit the same epidermal reactions as

1. Birth of skin lipids with polar detergents;
2. Physical junking of the stratum corneum with tenacious tape recording,
3. Chemically convinced vexation.

Have handed us our current understanding of the stratum corneum's function.

J B SALGAR INAMDAR SOFIYA TEJAL GANDHI AKANKSHA KADAWALE

Epidermis

The two main cell types that compose up the stratified, scaled epithelial subcaste that's the epidermis are dendritic cells and keratinocytes. Intercellular islands and riotous volumes of stainable cytoplasm distinguish keratinocytes from "clear" dendritic cells (5). Melanocytes, Langerhans cells, and Merkel cells are examples of novel cell populations, even though keratinocyte cells make up the majority of epidermal cell maturity. Depending on how keratinocytes look and where they are when they differentiate into wanton cells, the epidermis is usually divided into four layers.

Cornified or wanton cell subcaste (stratum corneum),

- Scaled cell subcaste (stratum spinosum),
- Grainy cell subcaste (stratum granulosum) and
- Rudimentary cell subcaste (stratum germinativum).
- The lower three layers of the epidermis, known as stratum malpighia or rete malpighia, contain living, nucleated cells.

Sweat glands, nails, and pilosebaceous appendages are examples of secondary structures that arise from the epidermis, a subcaste that regenerates continuously. The external epidermis can regenerate because the primitive cells of the epidermis initiate cycles of proliferation. The epidermis is a dynamic towel whose cells move in accord every time due to differences they approached the skin's face, colorful varieties of cells pass not only one another but also Langerhans and melanocytes.

Dermis

Collagen fibers, fibroblast cells, fat cells, blood vessels, nerve fibers, and touch receptors make up the dermis, the middle layer of the skin (4). There are two strata in the Dermis subcaste. The dermis is also known as the corium.

Reticular dermis. The lowest layer of the dermis is called the reticular subcaste. Blood vessels, glands, hair follicles, lymphatics, jitters, and fat cells are all part of its dense structure. A complex web of collagen and elastin fibers envelops the reticular dermis. Our skin may move and stretch thanks to these fibers, which also give it structural support.

Papillary dermis. Our dermis's top subcaste is the papillary subcaste. Compared to the reticular dermis, it is significantly thinner. Collagen fibers, fibroblast cells, fat cells, blood vessel whim-wham fibers, and touch receptors are its constituents. The papillary dermis extends to the basement subcaste epidermis subcaste. They form a strong bond that connects like interlocking fritters. Each subcaste of our skin works together to cover the body.

Hypodermis

The hypodermis is the skin's deepest layer, consisting of adipose tissue, blood vessels, and connective tissue (4). It acts as a reserve energy source, separates the body, guards against damage, and connects skin to muscle and bones.

The hypodermis subcaste includes:

Adipose towel is an adipose towel made up primarily of adipocytes.

Blood vasculature comprises freeways, capillaries, and modes. They circulate blood throughout the body, provide oxygen to critical organs, and eliminate waste.

Along with the other layers of skin, the hypodermis shields our muscles, organs, and skeleton from damage. The hypodermis acts as a reserve energy source, preserves the skin, and allows mobility by sliding over supporting structures.

Adipocytes, which are arranged into lobules characterized by stringy connective towel (septa), are the main ingredients of the hypodermis.

The septa include blood, lymphatic, and whim-wham vessels. Subcutaneous towel can store energy through three natural processes: endotrophic, deposit, and exotrophic.

Adipocytes under the skin have an intermediate called an adiponectin. Likewise, because it changes androstenedione into estrone, the subcutaneous towel's aromatase enzyme is believed to be an endocrine organ. Likewise, the hormone leptin, which regulates body weight, is produced by adipocytes.

Cream

Definition of cream. Creams are thick semisolid mixes that are meant for external operation. They generally contain a water-answerable base so that it can fluently be removed from the skin. When applied to the skin, creams leave on visible substantiation of their presence on skin. Cream has a fairly soft, spreadable thickness.

A cream is a topical treatment that's frequently applied topically to the skin. Creams are also used as topical treatments for mucous membranes, including the vaginal or rectum. As indeed ornamental creams are formulated using drugstore procedures and unmedicated creams are extensively employed in a range of skin conditions (dermatoses), creams could be classified as pharmaceutical particulars.

Skin care products. Creams are emulsions that are either water-in-oil or oil-in-water.

Skin cream ingredients include

An outline of the fundamental components required to create skin creams is given below. Water, vegetable and petroleum oils, fats and their derivatives, humectants, and emulsifiers are all included in this. One of the most common basic ingredients used to make cream is water.

Antibacterial cream

"Cream used to destroy or inhibit bacterial growth."

- Benefits of cream
  - Can decrease inflammation (1).
  - Improve our skin tone (4).
  - Helps prevent wrinkles and pimples (2).
  - Boosts blood circulation and cell metabolism (3)
  - Easily water-washable (5)
  - Simple to clean up after (1)
  - Less oily than ointment (4)
  - Suitable for fair, sensitive, and dry skin (2)
  - Easy to apply and spread across the skin's surface (3)
  - Perfect for recent injuries (5)
- Cream has the following drawbacks
  - Higher chance of contamination (1)
  - Lower viscosity than other semi-solid medications (4)
  - May cause skin irritation or contact dermatitis (2)
  - Certain medicines have low skin permeability (3).
  - Allergic reactions may occur (5).
  - Only suitable for drugs requiring low dosage (1).
  - Enzymes in the epidermis may denature drugs (4).
  - Larger particle size drugs are more difficult to absorb through the skin (6).

Bacterial skin infections

Bacterial skin infections frequently do when bacteria access the skin hedge through injuries, scrapes, nonentity mouthfuls, or preexisting skin conditions (1). Common bacterial pathogens include *Staphylococcus aureus* and *Streptococcus pyogenes* (4). Conditions such as cellulitis, impetigo, and abscesses are typical examples (2). Severe cases can lead to systemic complications like sepsis if untreated (5).

In the context of atopic dermatitis (AD), patients are particularly susceptible to bacterial infections due to compromised skin barriers and immune dysregulation (7). *S. aureus* colonization is prevalent in AD patients, exacerbating inflammation and increasing infection risk (8). Managing bacterial colonization in AD is challenging, as *S. aureus* can colonize even non-lesional skin, complicating diagnosis and treatment (9).

The reasons behind bacterial infections

Bacterial infections are brought on by harmful bacteria, which can enter the body in a number of ways and, in the right circumstances, start to colonize and grow (3).

The major bacterial causes of skin infections are shown in **Figure 2** and detailed in **Table 1**.

Risk factors for bacterial infections

Various intrinsic and extrinsic factors can increase the likelihood of bacterial infections:

- Intrinsic (patient-related) risk factors
  - Compromised immunity (e.g., diabetes, human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS), cancer) (1).
  - Poor hygiene (4).
  - Malnutrition (2).
  - Chronic skin conditions (eczema, dermatitis) (5).
  - Advanced age or infancy (3).
- Extrinsic (environmental or external) risk factors
  - Injuries, cuts, or burns (providing entry points) (9).
  - Invasive medical procedures (e.g., catheterization) (8).
  - Applying tainted medical supplies or dressings (7).
  - Direct interaction with sick people (2).
  - Warm, humid conditions that encourage the growth of bacteria (4).
- Pathophysiology of bacterial infections

i. Entry of pathogenic bacteria
Bacteria enter the body through:
  - Skin wounds or abrasions (1).
  - Respiratory tract (e.g., via inhalation) (5).
  - Gastrointestinal tract (contaminated food/water) (3).
  - Urogenital tract (2).
  - Medical instruments or catheters (8).

ii. Colonization
Bacteria use surface molecules (adhesins or fimbriae) to attach themselves to host tissues once they are inside. They may create biofilms to defend themselves

TABLE 1 | Causes of bacterial infections (1, 8, 9).

Bacterium	Associated infections
Staphylococcus aureus	Skin infections, abscesses, impetigo, methicillin-resistant *Staphylococcus aureus* (MRSA)
Escherichia coli	Wound infections, urinary tract infections
Pseudomonas aeruginosa	Burns, chronic wounds, diabetic ulcers
Streptococcus pyogenes	Cellulitis, impetigo, erysipelas
Propionibacterium acnes	Acne and folliculitis

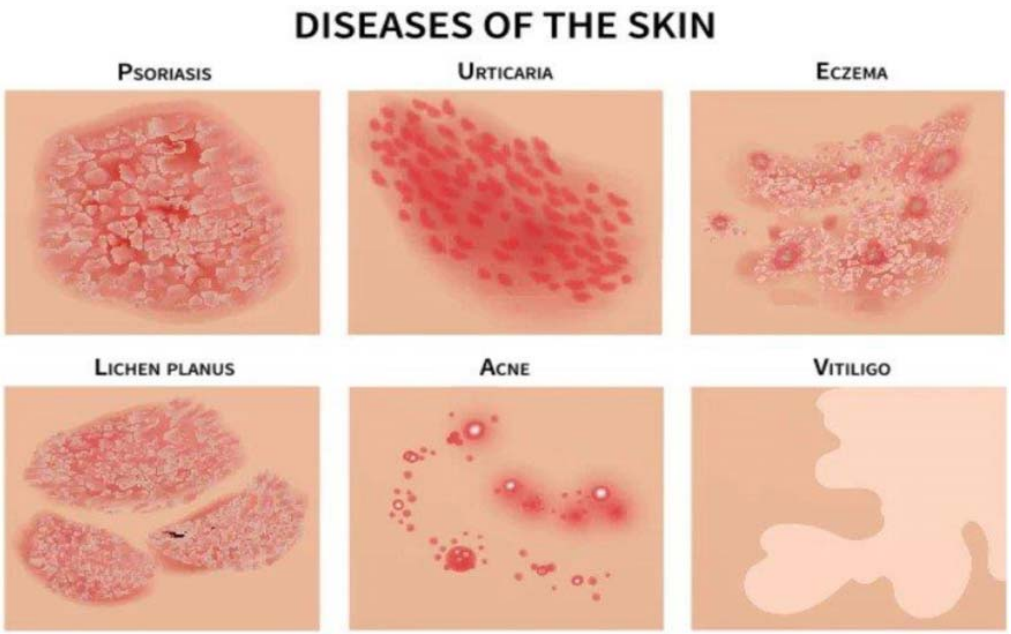


FIGURE 2 | Causes of bacterial skin infections (1, 4, 9).

against immunological attacks, particularly on skin or wounds (7).

iii. Multiplication
Bacteria grow quickly in an environment that is favorable to them. When bacterial proliferation surpasses immune defense, the host's immune system may become overpowered (4).

iv. Invasion and damage
Some bacteria release:

- **Toxins** (e.g., exotoxins or endotoxins) that damage host tissues (2).
- **Enzymes** like proteases, lipases, and hyaluronidases that help in spreading through tissues (5).

This leads to:

- Inflammation (1)
- Swelling and redness (4)
- Pus formation (8)
- Tissue necrosis in severe cases (3)

v. Immune response

- The body activates immune cells (neutrophils, macrophages) (5).
- Inflammatory mediators (cytokines, histamine) are released (2).
- Fever, pain, and swelling are common signs of immune activity (4).

The pathophysiological process of bacterial infections is illustrated in **Figure 3**.

Outcome on bacteria.

- Cell wall breakdown → cell rupture (1)
- Membrane disruption → ion leakage (4)
- Protein synthesis inhibition → stunted growth (2)
- deoxyribonucleic acid (DNA)/ribonucleic acid (RNA) damage → no replication (5)
- Reduced virulence/biofilm → easier eradication by immune system (7)

These findings are summarized in **Table 2**.

TABLE 2 | Herbal antibacterial mechanisms (10–13).

Plant	Key compounds	Antibacterial mechanism
Lantana camara	Lantadenes, flavonoids	Cell membrane damage, inhibition of deoxyribonucleic acid (DNA)/ribonucleic acid (RNA) synthesis
Piper betle	Eugenol, chavicol, tannins	Membrane disruption, protein denaturation
Ocimum sanctum	Eugenol, ursolic acid, rosmarinic acid	Membrane lysis, inhibition of quorum sensing

Emerging treatments and antibacterial resistance

Alternative therapies for skin infections are being investigated as a result of the increase in antibacterial resistance (8). Due to their broad-spectrum action and capacity to accelerate wound healing, antimicrobial peptides (AMPs) have shown promise (3). The goal of current AMP research is to create novel therapeutic medicines to fight resistant infections (2). Probiotics are also being researched for their potential to help treat skin disorders (4). Probiotics may aid in the treatment of conditions like eczema and acne

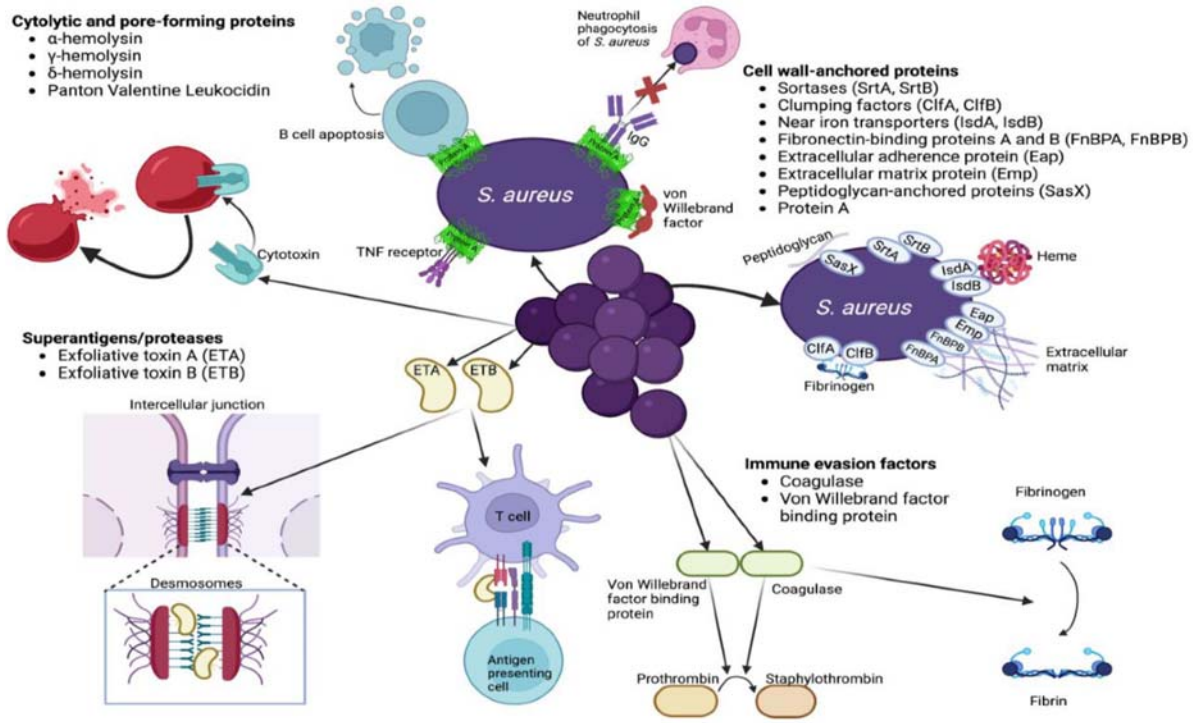


FIGURE 3 | Pathophysiology of bacterial infections (2, 4, 5).

by fostering a balanced microbiome, but further research is required to determine their effectiveness (1).

The purpose of the polyherbal cream, which contains ***Ocimum sanctum***, ***Piper betle***, and ***Lantana camara***, is to:

- **Inhibit bacterial growth** on skin (7).
- **Prevent biofilm formation** in wounds (8).
- **Promote healing** by reducing microbial load (5).
- **Offer a natural, side-effect-free alternative** to synthetic antibiotics (3).

Literature review

The escalating issue of Antibacterial resistance has intensified the search for alternative therapeutic agents. Herbal medicines, with their diverse bioactive compounds, have emerged as promising candidates. Polyherbal formulations, which combine multiple plant extracts, are particularly noteworthy due to their potential synergistic effects, enhanced efficacy, and reduced side effects. This chapter delves into the Antibacterial properties of *Lantana camara*, *Piper betle*, and *Ocimum sanctum*, and the rationale behind their combined use in topical formulations (10).

Lantana camara, belonging to the Verbenaceae family, is traditionally used for treating colourful affections (11). Its Antibacterial efficacity has been substantiated in several studies:

Antibacterial Activity: Ethanolic extracts of *L. camara* leaves have demonstrated significant inhibitory effects against pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The minimum inhibitory concentrations (MICs) observed were notably low, indicating potent antibacterial properties.

Phytochemical Constituents: The Antibacterial activity is attributed to compounds like lantadenes, flavonoids, and essential oils present in the plant (11).

Piper betle, commonly known as betel leaf, is renowned for its medicinal properties (12):

Broad-Spectrum Antimicrobial Activity: Extracts and essential oils from *P. betle* have shown inhibitory effects against a range of microorganisms, including Gram-positive bacteria like *Staphylococcus aureus*, Gram-negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, and fungi like *Candida albicans*. Notably, these extracts have also been effective against multidrug-resistant strains.

Synergistic Effects: Combining *P. betle* extracts with conventional antibiotics like streptomycin and gentamicin has resulted in enhanced antibacterial activity, suggesting a potentiating effect.

Active Compounds: The antimicrobial properties are primarily due to bioactive compounds such as eugenol, chavibetol, and hydroxychavicol (12).

Ocimum sanctum, or holy basil, is a staple in traditional medicine (13):

Antibacterial and Antifungal Activity: Aqueous and ethanolic extracts of *O. sanctum* have exhibited significant

antimicrobial activity against pathogens responsible for dental caries and other infections. For case, at attention of 5 and 10, Tulsi excerpts demonstrated antimicrobial exertion similar to doxycycline.

Essential Oils: The essential oil of *O. sanctum* contains compounds like camphor, eucalyptol, and eugenol, which have been shown to inhibit the growth of *Staphylococcus aureus* (including MRSA) and *Escherichia coli* (13).

Combining multiple herbal excerpts can lead to enhanced remedial actions (14):

Enhanced Antimicrobial Activity: Polyherbal creams formulated with various plant extracts have demonstrated superior antibacterial activity against pathogens like *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. These formulations also exhibited good stability and no adverse skin reactions.

Mechanism of Action: The synergistic effect in polyherbal formulations is believed to arise from the combined action of different bioactive compounds, leading to improved efficacy and reduced chances of microbial resistance (14).

The individual antimicrobial properties of *Lantana camara*, *Piper betle*, and *Ocimum sanctum* are well-documented. Their combination in a polyherbal cream formulation holds promise for enhanced antimicrobial efficacy, stability, and safety. This literature review underscores the potential of such formulations in addressing skin infections and contributing to the development of alternative antimicrobial therapies (15).

Methodology

Plant profile

The detailed plant profile is provided in **Table 3**.

Preparation of extracts (using heating mantle)

Using a heating mantle, the dried and powdered leaves of *Ocimum sanctum*, *Piper betle*, and *Lantana camara* were extracted ethanolically. The following is how the procedure was carried out:

1. Put the ground leaves in a flask with a round bottom.
2. Fill the flask with ethanol in the prescribed proportion.
3. Preheat the heating mantle to between 50°C and 60°C.
4. To guarantee uniform extraction, heat the mixture for three to four hours, stirring occasionally. Avoid temperatures above 60°C to prevent degradation of heat-sensitive bioactive compounds.
5. Allow the mixture to cool to room temperature.
6. Storage: After being weighed, the final concentrated extracts were placed in amber-colored glass containers and refrigerated at 4°C until they were needed for formulation.

Phytochemical screening of extracts

The results of phytochemical screening are shown in **Table 4** and **Figure 4**.

Formulation of polyherbal cream

Formulation table. The concentration range (0.9–1.8 g) was selected based on preliminary screening studies and aligns with commonly used extract doses in similar topical herbal preparations.

Table 5 shows the composition of various formulation batches.

Procedure

Preparation of oil phase (heated to 70–75°C):. In a clean beaker, accurately weigh and combine stearic acid, beeswax, stearyl alcohol, and liquid paraffin. Heat this mixture to 70–75°C with constant stirring until all components are fully melted.

The visual differences between formulations before adding the extracts are shown in **Figure 5**.

Preparation of active herbal extract phase (API phase). In a separate beaker, mix the ethanolic extracts of *Lantana camara*, *Piper betle*, and *Ocimum sanctum*. Stir gently at room temperature to maintain the integrity of the bioactive compound.

Preparation of emulsifier and preservative phase. In a third beaker, dissolve Tween 80 in glycerine as per batch quantity. Add methylparaben and stir the solution until fully homogeneous.

Emulsification process. Gradually add the emulsifier mixture into the oil phase while continuously stirring. Continue mixing until a uniform, creamy emulsion is formed.

Cooling and addition of herbal extracts (below 45°C). Allow the emulsion to cool to below 45°C. Slowly incorporate the herbal extract mixture while stirring constantly to avoid phase separation.

TABLE 3 | Plant profile (10–13).

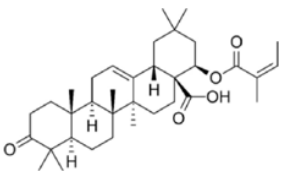
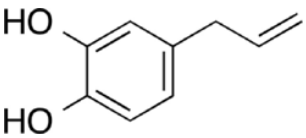
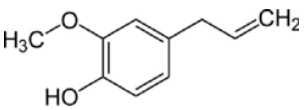
Parameter	*Lantana camara*	*Piper betle*	*Ocimum sanctum*
			
Synonyms	Wild Sage, Red Sage, Spanish Flag	Betel Leaf	Holy Basil, Tulsi
Scientific name	*Lantana camara* Linn.	*Piper betle* Linn.	*Ocimum sanctum* Linn. (*O. tenuiflorum*)
Biological source	Leaves and flowering tops of *L. camara*	Fresh or dried leaves of *P. betle*	Leaves and aerial parts of *O. sanctum*
Family	Verbenaceae	Piperaceae	Lamiaceae
Part used	Leaves and flowering tops (powder)	Leaves (fresh or dried, powder)	Leaves and aerial parts (powder)
			
Major constituents	**Lantadenes** A, B, C, and D (triterpenoids)	**Hydroxychavicol**, chavibetol, and eugenol	**Eugenol**, ursolic acid, and rosmarinic acid
Other phytochemicals	Flavonoids, tannins, and essential oils	Alkaloids, tannins, and saponins	Flavonoids, tannins, and essential oils
Structure of key compound	Lantadene A (Triterpenoid) 	Hydroxychavicol (Phenolic compound) 	Eugenol (Phenylpropanoid) 
Medicinal uses	Wound healing, anti-inflammatory, antibacterial, analgesic, and hepatoprotective	Oral hygiene, antiseptic, digestive aid, and wound healing	Adaptogen, immune booster, antibacterial, and stress relief
Other pharmacological effects	Antifungal (*Candida albicans*), anti-inflammatory, and antioxidant	Antioxidant, anti-inflammatory, and antifungal (*Candida* species)	Antiviral [e.g., herpes simplex virus (HSV), influenza], anti-inflammatory, and antioxidant

FIGURE 4 | Phytochemical analysis of extracts of *Lantana camara*, *Piper betle* & *Ocimum sanctum* respectively.

TABLE 4 | Phytochemical screening of extracts.

Test for	Name of the test	*Lantana camara*	*Piper betle*	*Ocimum sanctum*
Alkaloids	The Mayer's test	+	+	+
	The Wagner's test	+	+	+
	The Dragendoff's test	+	+	+
	The Hager's test	+	+	+
Flavonoids	The Shinoda test	+	+	+
Tannins	The test for the presence of Ferric chloride	+	+	+
Phenolic compound	The test for presence of Ferric chloride	+	+	+
Saponins	The Foam test	+	+	+
Cardiac glycosides	The Keller-Killiani test	+	+	+

Results and discussion

Evaluation of the polyherbal cream

Drug-excipient compatibility

These studies help identify any physical or chemical interactions between the active ingredients and excipients. Such interactions may influence the stability, efficacy, and safety of the final formulation. Conditions like temperature and humidity are used to simulate stress and evaluate potential incompatibilities.

Physical appearance

Results:

- Color: Greenish-brown
- Odor: Herbal fragrance
- Texture: Smooth and non-greasy (**Figure 6**).

pH determination

Using pH indicator paper, a drop of cream was placed and the resulting colour was compared to a standard chart (**Figure 7**).

Result: The pH ranged from 5.5 to 6.5, making it suitable for skin application.

Spreadability test

Spreadability was assessed using the parallel plate method:

The upper slide had a 20 g weight attached to it.

The time it took for the top slide to move 6 cm after cream was sandwiched between two glass slides was recorded.

The formula is as follows:

$$S = W * L/T$$

TABLE 5 | Formulation table.

Components	B1	B2	B3	B4	Uses
Lantana camara extract	0.9 g	1.2 g	1.5 g	1.8 g	Active pharmaceutical ingredient (API), Antibacterial, antifungal, wound healing
Piper betle extract	0.9 g	1.2 g	1.5 g	1.8 g	API, Antibacterial, antioxidant, anti-inflammatory
Ocimum sanctum extract	0.9 g	1.2 g	1.5 g	1.8 g	API, Antibacterial, anti-inflammatory, healing promoter
Stearic acid	1.5 g	1.5 g	1.5 g	1.5 g	Emulsifier, thickening agent
Liquid paraffin	2.4 g	2.4 g	2.4 g	2.4 g	Emollient, moisturizer, barrier protector
Beeswax	1.5 g	1.5 g	1.5 g	1.5 g	Thickening agent, skin protectant
Stearyl alcohol	0.9 g	0.9 g	0.9 g	0.9 g	Emollient, emulsifier, stabilizer
Tween 80 (Polysorbate 80)	0.6 g	0.6 g	0.6 g	0.6 g	Surfactant, emulsifier
Methylparaben	0.03 g	0.03 g	0.03 g	0.03 g	Preservative (prevents microbial growth)
Glycerine	7.67 g	7.67 g	7.67 g	7.67 g	Humectant (moisturizer), improves spreadability

FIGURE 5 | Formulation batches before adding API extracts.

FIGURE 6 | Image of polyherbal cream (batch 4 highest concentration).

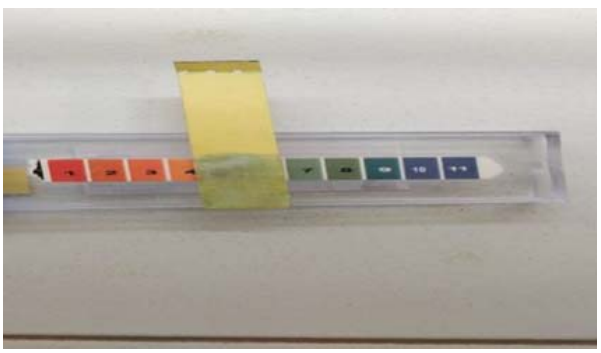


FIGURE 7 | pH determination.

Where,

S is the spreadability, W is the weight attached to the top slide, L is the slide length, and T is the spreading time.

$$S = \frac{W \times L}{T}$$

$$S = \frac{20 \times 6}{11}$$

$$S = 10.9 \text{ gm} \bullet \text{cm/sec}.$$

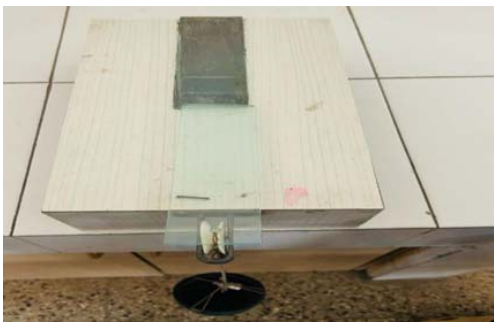


FIGURE 8 | Spreadability determination.

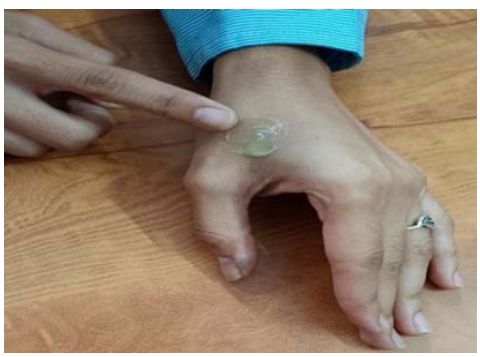


FIGURE 9 | Patch test.

FIGURE 10 | Standard cream.

A specific amount of cream was placed between two glass slides, and the spread diameter was measured (**Figure 8**).

Result: The cream exhibited good spreadability 10.9 g·cm/sec, indicating easy application.

FIGURE 11 | Antibacterial activity against *Staphylococcus aureus*.

TABLE 6 | Zone of inhibition against *Staphylococcus aureus*.

Sr. no.	Sample name	B3	B4	B5	B6	Control B2	Standard B1
1	**Zone of inhibition (in mm)**	14	13	-	-	11	15

FIGURE 12 | Antibacterial activity against *Escherichia coli*.

TABLE 7 | Zone of inhibition against *Escherichia coli*.

Sr. no.	Sample name	B3	B4	B5	B6	Control B2	Standard B1
1	**Zone of inhibition**	-	-	20 mm	14 mm	10 mm	20 mm

Homogeneity test

The uniformity of the preparation was assessed through touch and visual inspection.

Result: Homogeneous.

Patch test

A patch test was conducted on human volunteers to check for potential allergic reactions. The cream was applied to the forearm and observed after 48 h (Figure 9).

Result: Negative (−), no reaction, indicates no allergy to the substance tested.

Skin irritation

The cream was evaluated for its potential to cause skin irritation. No adverse reactions such as redness, itching, or inflammation were observed.

Result: Non-irritant.

Washability

A washability test determines how easily a material can be cleaned, particularly after being soiled or stained. It's used in various applications, including evaluating the effectiveness of cleaning agents, assessing the durability of coatings, and even measuring how well a coal sample can be separated from rock and minerals.

Result: Easily washable.

Antibacterial activity

Microorganisms used.

Gram-positive *Staphylococcus aureus*
Gram-negative *Escherichia coli*.

Method: agar well diffusion.

1. **Inoculation:** Bacterial cultures were swabbed onto nutrient agar plates.
2. **Well Preparation:** The agar was divided into 8 mm diameter wells.
3. **Sample loading:** Herbal cream extract solutions were added to each well. Standard Cream "Grab"(10 µg/disc) was used as a standard for comparison.

   The standard cream contains Nimba (*Azadirachta indica)* – 15%, Daruharidra (*Berberis aristata*) – 10%, Yastimadhu (*Glycyrrhiza glabra*) – 10%, Sariva (*Hemidesmus indicus*) – 10%, Swetha Kutaja (*Wrightia tinctoria*) – 10%, Durva (*Cynodon dactylon*) – 10%, Haridra *(Curcuma longa*) – 5%, Pancha Valkala (Group of five barks) – 10%, Karanja oil (*Pongamia glabra*) – 10%, Jashada Bhasma (Classical Prepn) – 5% (Figure 10).
4. **Incubation:** The plates were incubated for 24 h at 37°C.
5. **Observation:** To assess the efficacy of the antibiotic treatment, zones of inhibition were measured surrounding every well.

Results:

A. Antibacterial activity against *Staphylococcus aureus* (Figures 11 and 12)

B. Antibacterial activity against *Escherichia coli* (Tables 6 and 7)

Comparison of antibacterial activity.

- Against *Staphylococcus aureus* (Gram-positive): B3 outperformed the control (11 mm) and displayed the largest inhibitory zone (14 mm) against *S. aureus*, which was nearly equal to the standard (15 mm).
- Against *Escherichia coli* (Gram-negative): B5 showed excellent activity (20 mm) against *E. coli*, equal to the standard drug (20 nm), and better than the control (10 nm).
- When it comes to treating infections brought on by Gram-positive bacteria (*Staphylococcus aureus*), batch B3 is the most effective.
- Batch B5 is the best for Gram-negative infections (*Escherichia coli*).
- The superior performance of B3 against *S. aureus* may be attributed to its optimal extract concentration, which appears to enhance efficacy against Gram-positive strains. In contrast, B5's increased concentration of active phytoconstituents likely contributed to its enhanced Gram-negative activity.

Conclusion

The present study successfully formulated and evaluated a polyherbal cream containing *Lantana camara*, *Piper betle*, and *Ocimum sanctum*, targeting its potential as a safe and effective topical antibacterial agent. The cream's antibacterial effectiveness against both Gram-positive and Gram-negative bacteria, skin compatibility, and physicochemical characteristics were evaluated. The preparation demonstrated favorable organoleptic and physical characteristics, including a greenish-brown color, smooth non-greasy texture, herbal fragrance, and appropriate pH range (5.5–6.5), making it suitable for application on human skin. The cream showed good spreadability (10.9 g/sec) and excellent washability, enhancing its user convenience and compliance. Among other safety evaluations, patch testing and skin irritation tests confirmed that the formulation is non-irritating and appropriate for topical use. This demonstrates that it is appropriate for usage on delicate or weakened skin conditions that are frequently impacted by bacterial infections.

The agar well diffusion method of antimicrobial assessment produced encouraging findings:

- The efficacy of batch B3 against Gram-positive bacteria was demonstrated by its robust activity against *Staphylococcus aureus* (14 mm zone of inhibition), which was nearly identical to that of the conventional medication (15 mm).
- Batch B5's potency against Gram-negative bacteria was confirmed by its outstanding suppression of *Escherichia coli* (20 mm), which was comparable to the standard.

The selective antibacterial activity found indicates that the formulation can be modified for a specific therapeutic purpose, even though no single batch demonstrated broad-spectrum action. The presence of potent phytoconstituents in the selected herbs is likely responsible for the observed antibacterial effects.

Ethics statement

All procedures involving plant extract formulation and microbiological testing were carried out in compliance with institutional research ethics and safety guidelines.

Author contributions

JBS: Supervision, Writing – review and editing. SSI: Investigation, Data curation, Writing – original draft, Project administration. TPG: Methodology, Formal analysis, Resources. SCK: Laboratory work, Validation, Visualization. AAK: Literature review, Data analysis, Writing – review and editing. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Abbreviations

API, active pharmaceutical ingredient; AD, atopic dermatitis; AMP, antimicrobial peptides; CFU, colony forming unit; OH, ethanol; MIC, minimum inhibitory concentration; pH, potential of hydrogen; MRSA, methicillin-resistant *Staphylococcus aureus*.

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