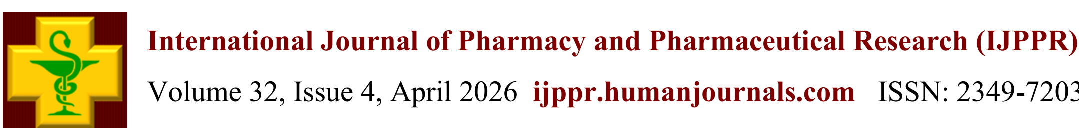
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In Silico and In Vitro Antibacterial Activity of *Syzygium aromaticum* (Clove) Against Bacterial Implicated in Impetigo Skin Disease

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ABSTRACT

Impetigo is a highly contagious superficial bacterial skin infection primarily caused by Staphylococcus aureus and Streptococcus pyogenes, commonly affecting children and spreading rapidly under poor hygienic conditions. The increasing prevalence of antimicrobial resistance has reduced the effectiveness of conventional antibiotics, highlighting the need for alternative therapeutic agents. The present study aims to evaluate the antibacterial potential of *Syzygium aromaticum* (L.)against bacteria responsible for impetigo using both in vitro and in silico approaches. Clove buds were subjected to Soxhlet extraction to obtain clove oil, and preliminary phytochemical screening confirmed the presence of phenols, flavonoids, glycosides, and carbohydrates. The antibacterial activity of the extract was assessed against Staphylococcus aureus using the agar well diffusion method, and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The extract demonstrated significant antibacterial activity with measurable zones of inhibition, indicating its effectiveness against Gram-positive pathogens associated with impetigo. In addition, molecular docking studies were performed to evaluate the interaction of eugenol, the major bioactive compound of clove, with the target bacterial protein (PDB ID: IVQQ). Docking analysis revealed favorable binding affinity and stable interactions, suggesting a potential mechanism for its antibacterial activity. The findings of this study suggest that clove possesses promising antibacterial properties and may serve as a potential natural alternative or adjunct therapy for the management of impetigo. Further in vivo and clinical studies are recommended to validate its therapeutic applicability and safety.

Keywords: *Syzygium aromaticum* (L.), Impetigo, Staphylococcus aureus, Antibacterial activity, Eugenol and Antibiotic resistance.

1. INTRODUCTION

1.1. Skin

Skin also called the integumentary system, is the largest organ of the human body and forms the outer protective covering. It serves as the body's first physical barrier against the external environment. In an average adult, the skin covers an area of about 2 m² and weighs approximately 4.5–5 kg, accounting for 12–15% of total body weight. Structurally, the skin is composed of three main layers: the epidermis (outer layer), the dermis (middle supportive layer), and the subcutaneous tissue (innermost layer). Each layer differs in structure, nocomposition, and function [1].

1.1.1. Structure of skin

a) Epidermis

The epidermis, the outermost layer of the skin, serves as a protective barrier against the external environment. It effectively shields the body from harmful stimuli while also functioning as a semipermeable membrane that helps maintain internal moisture balance. Compared to other terrestrial mammals, the human epidermis is relatively thicker and more robust [2].

b) Dermis

The dermis is the central layer of the skin, located between the epidermis and the hypodermis. It is thicker than the epidermis and contains blood vessels and nerves that support and nourish the outer layer. The dermis is divided into two regions: the superficial papillary layer and the deeper reticular layer (figure 1) [1].

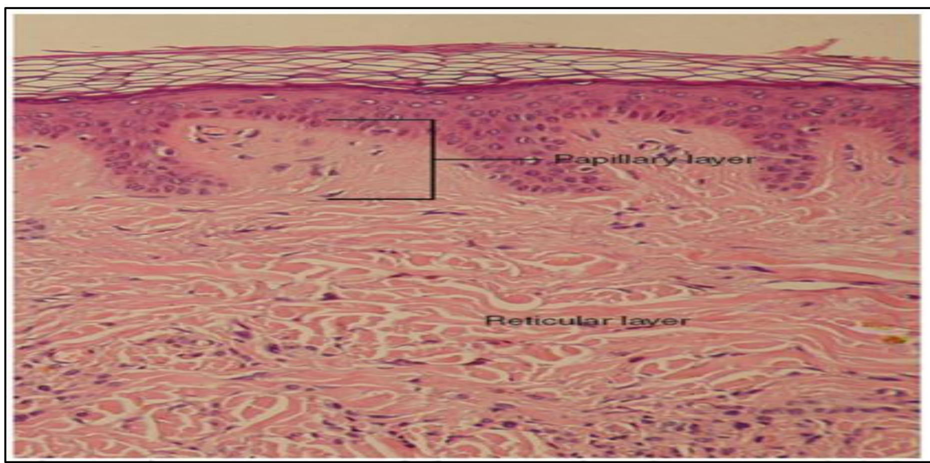

Figure 1:Histology of the skin showing the different sections of the dermis: the papillary layer and the reticular layer [1].

The dermis also contains important structures such as sweat glands, hair follicles, muscles, sensory nerve endings, and blood vessels [3].

c) Hypodermis

The hypodermis, also called the subcutaneous fascia, is the deepest layer of the skin and is situated below the dermis. It contains adipose (fat) lobules, blood vessels, sensory nerve endings, and a small number of skin appendages such as hair follicles [3].

1.1.2. Function of the skin

a) Protection

The skin primarily serves as a barrier, protecting the body from microorganisms and harmful substances. Its immunity relies on resident cells like Langerhans cells and melanocytes, as well as circulating immune cells such as neutrophils and macrophages. Aging reduces the number and efficiency of these cells, weakening the skin's protective function.

b) Sensation

The skin protects the body from the external environment through a sensory system that responds to stimuli, such as cold. All skin layers epidermis, dermis, and hypodermis contain specialized nerve structures. Meissner corpuscles, mainly in the fingertips, detect light touch, while deeper Vater-Pacini corpuscles sense pressure.

c) Regulation of the body temperature

The skin helps maintain a body temperature of 37°C through vasodilation and vasoconstriction. When temperature drops, dermal arterioles constrict, sweating stops, and hairs stand up to retain heat. When temperature rises, sweat glands produce sweat for cooling, and arterioles dilate to release excess heat.

d) Immunological function

Human skin contains both innate and adaptive immune responses. Interaction between skin cells, like Langerhans cells, and immune cells helps defend against microorganisms and prevent infections.

e) Endocrine function

The skin produces various hormones, including steroids and vitamin D. Vitamin D is synthesized in the epidermis when exposed to UV radiation and is essential for the absorption of calcium and phosphorus, the main components of bones[1].

1.1.3. Types of skin barrier

a) Physical:

This is the first line of defense, created by the tight packing of corneocytes (skin cells) that are cemented together by a lipid matrix.

Corneocytes: 66 These are the “bricks” of the barrier.

Lipid matrix: This is the “mortar” that holds the corneocytes together and is composed of ceramides, cholesterol, and free fatty acids.

b) Chemical:

This barrier is maintained by the skin’s slightly acidic pH and the presence of antimicrobial activity.

The acidic mantle helps protect against harmful bacteria.

c) Microbial:

The skin is home to a variety of microorganisms, and the skin barrier helps maintain a balance of these microbes. The barrier releases antimicrobial peptides that protect against pathogens.

d) Immunological:

The epidermis can mount an immune response, which is crucial for wound healing and protecting the body from infection after injury.

e) Neuronal:

The epidermis contains sensory nerves that allow the skin to feel and react to stimuli.

1.2.Types Of Skin Infections

a) Bacterial infections

Bacterial infections are the most common skin issues in HS(Hidradenitis suppurativa) athletes, primarily caused by Staphylococcus and Streptococcus species, leading to conditions like impetigo, erysipelas, cellulitis, folliculitis, and abscesses.

b) Fungal infections

Fungal infections are the second most common skin issue in HS athletes, affecting the scalp (tinea capitis), body (tinea corporis), groin (tinea cruris), feet (tinea pedis), or nails (onychomycosis). Key dermatophytes include Trichophyton rubrum, T. tonsurans, and Microsporum canis, with T. tonsurans causing most cases, including 90% of urban tinea capitis. Studies show tinea accounts for over 70% of skin infections in wrestling tournaments, affecting 19–40% of participants each season.

c) Herpes infections

Herpes infections in athletes include varicella (chickenpox) and herpes simplex virus (HSV) infections. Chickenpox is now rare due to vaccination and causes fever, lymphadenopathy, and a widespread vesiculopapular rash. HSV, more common in contact-sport athletes, is usually localized to the head, neck, and face, sometimes causing sore throat. HSV-1 accounts for 94–97% of cases, often affecting the face in wrestlers and occasionally the eyes, risking corneal scarring or blindness. Transmitted via direct skin-to-skin

contact, HSV outbreaks in wrestlers—known as herpes gladiatorum—occur mainly on areas of frequent contact, unlike non-athletes where labial infections dominate.

d) Viral infections

Molluscum contagiosum, a Poxviridae virus, spreads via skin-to-skin contact and appears as 2–10 mm dome-shaped papules with central depressions, usually without redness. Lesions affect the groin in adults and the neck, chest, axilla, and arms in children and wrestlers. Treatment includes curettage, hyfrecation, or topical 5% imiquimod. Facial lesions need careful management to prevent scarring. Athletes can return to contact sports once treated and properly covered [4].

1.2.1. Epidemiology of Skin Infection

Assessing the incidence and prevalence of skin and soft tissue infections (SSTIs) is challenging due to their variable presentation. The estimated incidence is 24.6 per 1,000 person-years, while prevalence is difficult to determine because most cases resolve within 7–10 days. SSTIs account for 7%–10% of all hospitalized patients, and they are the third most common diagnosis in emergency departments, following chest pain and asthma. They occur more frequently in men (60%–70%) and in individuals aged 45–64 years. About 70%–75% of cases are treated in the outpatient setting, commonly involving the lower leg. Severe complications are uncommon, with low rates of erysipelas (0.09/1000 person-years), lymphadenitis (0.16%), lymphangitis (0.16/1000 person-years), and necrotizing fasciitis (0.04/1000 person-years) [5].

1.3. Impetigo

Impetigo is a highly contagious bacterial skin infection primarily caused by Staphylococcus aureus and Streptococcus pyogenes. It is most common in children aged two to five, although it can affect all age groups. The condition frequently arises after minor skin trauma and is associated with factors such as hot and humid climates, poor hygiene, crowding, malnutrition, and medical conditions like diabetes. Impetigo often spreads through autoinoculation via fingers, towels, or clothing and can easily pass to close contacts. While usually self-limited, antibiotics are used to speed recovery and reduce transmission. Preventive measures include good hygiene, handwashing, regular bathing, and proper care of minor wounds [6].

1.3.1. Types of Impetigo

a) Non-bullous impetigo

Non-bullous impetigo, or school sores, is a common bacterial skin infection mainly caused by Staphylococcus aureus and Streptococcus pyogenes. It is typically treated with topical or oral antibiotics such as mupirocin, fusidic acid, flucloxacillin, and clarithromycin. However, antibiotic resistance is increasing globally, making treatment more challenging [7].

b) Bullous impetigo

Bullous impetigo is a common skin infection in young children (ages 2–5) caused almost exclusively by coagulase-positive Staphylococcus aureus and related to staphylococcal toxin production. It typically presents with flaccid blisters and bullae under 3 cm, which may rupture to form superficial crusts. Diagnosis is usually straightforward from characteristic bullae and erosions, though atypical cases may mimic other conditions, such as thermal burns[8].

1.3.2. Etiology

Impetigo represents about 10% of pediatric skin complaints and is most common in children aged 2–5 years, though it can occur at any age. Overall incidence is similar in males and females, but adult men are more often affected. Cases peak in summer and fall. Bullous impetigo is more frequent in infants, with children under two years accounting for 90% of these cases[9].

1.3.3.Pathophysiology

Impetigo is classified as primary, occurring on previously healthy skin, or secondary, developing at sites of skin damage. Breaks in the skin—caused by trauma, insect bites, burns, varicella, dermatitis, or surgery—allow Staphylococcus aureus or Group A Streptococcus to invade. Self-inoculation can spread lesions. Risk increases with malnutrition, immunosuppression, diabetes, overcrowding, daycare exposure, and poor hygiene.

Common triggers that damage skin include:

- Varicella
- Herpes
- Scratching
- Lice
- Burns
- Trauma
- Insect bites [9].

1.3.4. Diagnosis

The diagnosis of both nonbullous and bullous impetigo is primarily clinical. The differential diagnosis includes several other blistering and rash conditions (table 1).

Skin swabs are not reliable for distinguishing true infection from bacterial colonization. If initial treatment is unsuccessful, culturing pus or fluid from ruptured bullae rather than intact skin can help identify the organism and its antibiotic sensitivity. Serologic tests for streptococcal antibodies are useful for diagnosing poststreptococcal glomerulonephritis, but they are not helpful for diagnosing impetigo [9].

Table 1: Differential Diagnosis of Impetigo [9]

Bullous	Nonbullous
Bullous erythema multiforme	Atopic dermatitis
Bullous fixed drug eruption	Bockhart impetigo*[10]
Bullous lupus erythematosus	Childhood discoid lupus erythematosus
Bullous pemphigoid reactions	Contact dermatitis
Bullous scabies	Cutaneous candidiasis
Contact dermatitis	Dermatophytosis (tinea corporis or capitis)
Dermatitis herpetiformis	Herpes simplex virus
Insect bites	Pediculosis (lice)
Linear immunoglobulin A bullous dermatosis	Scabies
Necrotizing fasciitis	Sweet syndrome (acute febrile neutrophilic dermatosis)
Pemphigus vulgaris	Varicella zoster virus
Stevens-Johnson syndrome	
Thermal burns	
Transient neonatal pustular melanosis	

1.3.5. Complications and Treatment

a) Complications

- **More extensive infections** such as cellulitis, lymphangitis, or bacteraemia.
- **Staphylococcal scalded skin syndrome.**

- **Scarlet fever.**

- **Post-streptococcal glomerulonephritis**an uncommon acute kidney disorder occurring 2–6 weeks after infection with Streptococcus pyogenes (group A streptococcus), caused by a type III hypersensitivity reaction.

- **Streptococcal toxic shock syndrome** a rare but serious condition characterised by widespread erythematous rash, fever, and low blood pressure.

- **Post-inflammatory hyperpigmentation.**

- **Scarring, which is more likely in cases of ecthyma.**

b) Treatment

Treatment options for impetigo include topical and systemic antibiotics, as well as topical antiseptics. However, high-quality evidence identifying the most effective therapy is limited. A 2012 Cochrane review analyzing 68 randomized controlled trials found no single treatment superior to others.

Topical antibiotics are generally more effective than placebo and preferred for localized disease, while systemic antibiotics are used for widespread or severe infections. In some cases, clinicians may combine topical and oral therapy.

An ideal treatment should be effective, affordable, safe, and should not contribute to antibiotic resistance [9].

1) Topical antibiotics

Topical antibiotics are applied directly to the infected area, helping reduce systemic side effects and antibiotic resistance. A seven-day treatment course is generally effective for impetigo.

However, possible drawbacks include local allergic reactions, skin irritation, and difficulty applying near sensitive areas like the eyes or mouth. Commonly recommended topical agents are mupirocin 2%, retapamulin 1%, and fusidic acid (not available in the U.S.).

Increasing resistance, including MRSA and mupirocin-resistant strains, has influenced treatment choices.

Retapamulin is a newer pleuromutilin antibiotic with strong activity against gram-positive bacteria and a lower likelihood of resistance development.

Approved by the FDA in 2007, it is indicated for impetigo caused by methicillin-susceptible S. aureus or S. pyogenes in patients nine months and older, but not for MRSA or intranasal use.

Mupirocin is generally more affordable than the newer, brand-only retapamulin ointment (table 2) [9].

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Table 2:Topical Antibiotics for Impetigo[9]

Table 2. Topical Antibiotics for Impetigo

Medication	*Instructions*	*Cost**
Fusidic acid 2% ointment†[17]	Apply to affected skin three times daily for seven to 12 days	Available in Canada and Europe
Mupirocin 2% cream (Bactroban)‡[8,18]	Apply to affected skin three times daily for seven to 10 days; reevaluate after three to five days if no clinical response Approved for use in persons older than three months	15-g tube: $48 ($89) 30-g tube: $50 ($144)
Mupirocin 2% ointment‡[11]	Apply to affected skin three times daily for seven to 14 days Dosing in children is same as adults Approved for use in persons older than two months	22-g tube: $14 ($103)
Retapamulin 1% ointment (Altabax)§[19]	Apply to affected skin twice daily for five days Total treatment area should not exceed 100 cm^2 in adults or 2% of total body surface area in children Approved for use in persons nine months or older	15-g tube: NA ($130) 30-g tube: NA ($245)

NA = not available.

**—Estimated retail price based on information obtained at http://www.goodrx.com (accessed April 7, 2014). Generic price listed first; brand listed in parentheses.*

†—Coverage for Staphylococcus aureus *(methicillin-susceptible) and streptococcus.*

‡—Coverage for S. aureus *(methicillin-susceptible) and streptococcus. Mupirocin-resistant streptococcus has now been documented.[6,14]*

§—First member of the pleuromutilin class of antibiotics. Coverage for S. aureus *(methicillin-susceptible) and streptococcus.[19]*

Information from references 6, 8, 11, 14, and 17 through 19.

2) Oral antibiotics

Oral antibiotics are recommended for impetigo with extensive lesions or when topical treatment is impractical(table 3). A seven-day course is usually adequate, with extension based on clinical response and culture results.

Studies show no clear advantage among oral antibiotic classes, and cure rates are similar between topical and oral therapy. Due to rising resistance, erythromycin and penicillin are no longer routinely recommended, and penicillin V is often ineffective.

Macrolides are also less preferred because of increasing resistance. Amoxicillin-clavulanate is more effective than amoxicillin alone due to β-lactamase coverage.

Cephalosporins are an option, although no generation has proven superior. Clinicians should consider local resistance patterns when selecting therapy[9].

Table 3: Systemic antibiotics for impetigo[9]

Table 3. Systemic Antibiotics for Impetigo

Drug	Adult seven-day dose	Cost (for a typical course of treatment)*	Children seven-day dose	Cost*
Amoxicillin/ clavulanate (Augmentin)†	875/125 mg every 12 hours	$19 ($193)	Younger than three months: 30 mg per kg per day Three months or older: 25 to 45 mg per kg per day for those weighing less than 40 kg (88 lb); 875/125 mg every 12 hours for those weighing 40 kg or more Based on mg per kg per day of the amoxicillin component in divided doses every 12 hours	1 bottle, 400/57 mg per 5 mL (100-mL oral suspension): $30 ($125)
Cephalexin (Keflex)	250 mg every six hours or 500 mg every 12 hours	$5 ($90)	25 to 50 mg per kg per day in divided doses every six to 12 hours	1 bottle, 250 mg per 5 mL (100-mL oral suspension): $14 (NA)
Clindamycin‡	300 to 600 mg every six to eight hours	$18 ($200)	10 to 25 mg per kg per day in divided doses every six to eight hours	1 bottle, 75 mg per 5 mL (100-mL oral solution): $47 (pricing varies by region)
Dicloxacillin	250 mg every six hours	$14 (NA)	12.5 to 25 mg per kg per day in divided doses every six hours	See adult pricing: no liquid formulation available
Doxycycline§	50 to 100 mg every 12 hours	$15 ($95)	2.2 to 4.4 mg per kg per day in divided doses every 12 hours Not recommend in children younger than eight years	1 bottle, 25 mg per 5 mL (60-mL oral suspension): $20 (pricing varies by region)
Minocycline (Minocin)§	100 mg every 12 hours	$36 ($185)	Loading dose of 4 mg per kg for first dose (maximum dose of 200 mg), then 4 mg per kg per day in divided doses every 12 hours Maximum of 400 mg per day Not recommend in children younger than eight years	See adult pricing: no liquid formulation available
Trimethoprim/ sulfamethoxazole§	160/800 mg every 12 hours	$4 (NA)	8 to 10 mg per kg per day based on the trimethoprim component in divided doses every 12 hours	1 bottle, 40/200 mg per 5 mL (100-mL oral suspension): $4 (pricing varies by region)

NOTE: *Because of emerging resistance, penicillin and erythromycin are no longer recommended treatments.*[12]

NA = not available.

**—Estimated retail price based on information obtained at http://www.goodrx.com (accessed April 7, 2014). Generic price listed first; brand listed in parentheses.*

†—Good coverage for Staphylococcus aureus *(methicillin-susceptible) and streptococcus.*

‡—If methicillin-resistant S. aureus *is suspected or proven.*

§—If methicillin-resistant S. aureus *is suspected or proven. There is no activity against streptococcus.*

Information from references 12 and 15.

1.4. Antibiotic resistance, recurrence, and side effects

a) Antibiotic resistance

Antimicrobial resistance (AMR) significantly affects infection outcomes and is projected to cause 10 million deaths annually by 2050, exceeding deaths from cancer and other major causes. AMR brings heavy economic and personal costs, estimated at up to 100 trillion USD. Global antibiotic use rose by nearly 40% from 2000–2010, leading to geographic differences in resistance patterns. With international travel accelerating the spread of resistant pathogens, a coordinated global response is essential. Recent expert panels on impetigo treatment raised concerns about AMR trends and the need for stewardship in topical therapies[10].

b) Recurrence

Having impetigo once does not provide immunity, so reinfection can occur. Recurrence rates range from 7% to 45% and may be linked to poor hygiene, underlying skin conditions like eczema, or infection with antibiotic-resistant strains such as MRSA. Preventive measures include good hygiene practices regular hand washing, short nails, and daily bathing and, for repeated infections, decolonization methods such as dilute bleach baths[11].

c) Side effects

Side effects vary depending on whether topical or oral antibiotics are used.

Topical antibiotics (mupirocin, fusidic acid, ozenoxacin) may cause mild itching, burning, or redness, and less commonly allergic contact dermatitis, particularly with older agents like neomycin and gentamicin.

Oral antibiotics (flucloxacillin, cephalexin, erythromycin) commonly lead to gastrointestinal symptoms such as nausea, vomiting, and diarrhea, and may occasionally cause skin rashes. Broader-spectrum oral agents also carry a risk of Clostridioides difficile infection[12].

1.5. Need for alternative therapies

Oral antibiotics are used for impetigo with extensive bullae or when topical treatment is unsuitable. Effective options include amoxicillin–clavulanate, dicloxacillin, cephalexin, clindamycin, doxycycline, minocycline, trimethoprim–sulfamethoxazole, and macrolides (penicillin is ineffective). Natural remedies like tea tree oil and Manuka honey lack solid evidence. New therapies include ozenoxacin and minocycline foam. Topical disinfectants are inferior and should not be used. Increasing resistance such as MRSA and mupirocin-resistant strains impacts treatment choices: fusidic acid, mupirocin, and retapamulin treat MSSA and streptococci; clindamycin targets MRSA; trimethoprim sulfamethoxazole covers MRSA but not streptococci [6].

1.6. Natural therapies

Evidence remains insufficient to clearly support or refute the use of herbal treatments for impetigo. Natural options like tea tree oil, tea extracts, olive, garlic and coconut oils, and Manuka honey have shown anecdotal benefit, but impetigo's self-limiting nature may make many remedies seem effective. One trial found comparable cure rates between tea leaf ointment and oral cephalexin (81% vs. 79%), and tea tree oil was reported to be similarly effective to mupirocin 2% for MRSA decolonization [6].

Importance of Medicinal herbs

- Natural Healing & Traditional Medicine
- Source of Modern Medicines
- Fewer Side Effects Compared to Synthetic Drugs
- Boosts Immunity & Prevents Diseases
- Affordable & Accessible Healthcare
- Supports Mental Health & Well-being
- Sustainable & Environmentally Friendly
- Holistic Approach to Health

1.7. *Syzygium aromaticum* (L.)

Syzygium aromaticum (L.)a member of the Myrtaceae family, originates from Indonesia and is now cultivated globally for culinary, medicinal, and aromatic uses. It is widely applied as a natural food preservative due to its strong antimicrobial activity. In addition, clove demonstrates antioxidant, analgesic, anesthetic, anti-inflammatory, and insecticidal properties, and its antioxidant potential contributes to the prevention of degenerative diseases. Traditionally, clove bud oil has been used to promote healing of wounds and burns[13].

Mechanism of antibacterial action of clove

Clove essential oil exhibits antibacterial action mainly by disrupting the bacterial cell membrane, resulting in cell death. Its mechanism includes:

(i) damaging the cell wall and membrane, causing leakage of cellular contents; (ii) penetrating the cytoplasm and affecting internal components; and (iii) inhibiting essential processes like DNA and protein synthesis(figure 2). This multi-step action highlights its strong molecular-level effectiveness as a natural antimicrobial agent [14].

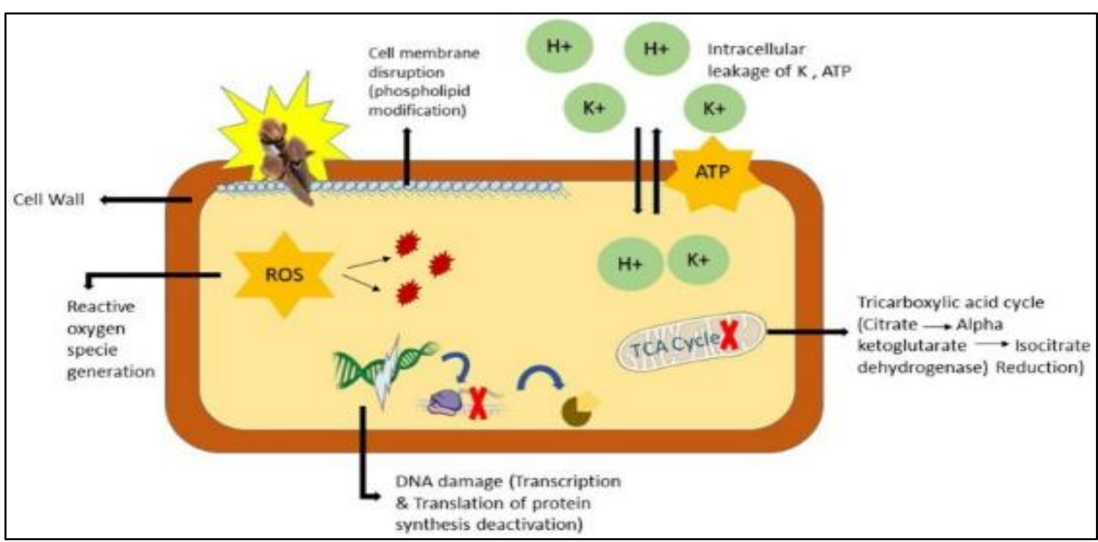

Figure 2: Mechanism of antibacterial action of clove [14]

1.8. Molecular Docking

Molecular docking is a computer-based technique used to predict how two molecules interact with each other typically a small drug-like molecule (ligand) and a biological target such as a protein or enzyme (receptor). The goal is to estimate:

The **binding orientation** (pose) of the ligand within the receptor's active or binding site, and

The **binding affinity**, representing how strongly they interact.

How Docking Works

1. Preparation of Ligand and Receptor

- Ligand: A 3D structure of the drug candidate is generated, geometrically optimized, and assigned proper atomic charges.
- Receptor: The protein structure is commonly downloaded from the Protein Data Bank (PDB), and unnecessary components like water molecules or co-crystallized ligands are removed.
- Active site detection: The binding pocket is identified either through known experimental data or by predictive computational tools.

2. Docking Procedure

Docking programs search for different possible ligand conformations and orientations within the receptor's active site. Various search algorithms, such as Monte Carlo methods or genetic algorithms, are used to simulate how well the ligand fits into the binding pocket.

3. Scoring Function

Each possible pose is scored based on estimated binding energy (expressed in kcal/mol). A lower energy score suggests a more favorable and stronger interaction. Scoring functions take into account:

- Hydrogen bonding
- Hydrophobic contacts
- Electrostatic interactions
- Van der Waals forces

4. Ranking and Interpretation

The ligand poses with the lowest binding energies are selected as the most promising and analyzed further. Visualization tools such as PyMOL, Discovery Studio, and LigPlot are used to examine molecular interactions.

1.8.1. Purpose and Applications

- Drug discovery: Used to identify new molecules that can strongly bind to targets associated with diseases.
- Lead optimization: Helps in modifying existing compounds to enhance their binding efficiency and interaction quality.
- Mechanistic insights: Provides understanding of how a ligand interacts with a target protein at the molecular level.
- Virtual screening: Enables rapid screening of large compound libraries to shortlist promising candidates.

1.8.2. Output Parameters

- Binding energy (ΔG): Indicates the stability of the ligand–receptor complex, expressed in kcal/mol (e.g., –7.8 kcal/mol); more negative values suggest stronger binding.
- Inhibition constant (Ki): Reflects the potency of the compound; lower Ki values correspond to higher inhibitory effectiveness.
- Interaction profile: Includes details such as the number of hydrogen bonds and the specific amino acid residues involved in binding.

1.9. Invitro antibacterial assay:

An in vitro antibacterial assay is a laboratory test performed outside a living organism (e.g., in test tubes, petri dishes, or microplates) to evaluate the ability of a substance—such as plant extract, essential oil, synthetic drug, or chemical compound—to inhibit the growth of bacteria or kill them.

Purpose:

The main purposes of in vitro antibacterial assays are:

- To determine the antibacterial activity of a test compound against specific pathogenic bacteria.
- To evaluate bacteriostatic or bactericidal effects (whether a substance stops bacterial growth or kills bacteria).
- To measure the concentration needed to inhibit bacterial growth, such as MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration).

- To screen potential antibacterial agents before animal or clinical studies.
- To compare effectiveness between different natural extracts, drugs, or formulations.

2. PLANT PROFILE

2.1.*Syzygium aromaticum* (L.)

Syzygium aromaticum (L.) is the dried flower bud of a Myrtaceae plant native to Indonesia's Maluku Islands, now cultivated worldwide. The tree begins producing commercial buds after four years, which are harvested by hand or using phytohormones. Clove is widely valued for its medicinal, aromatic, and preservative properties. Owing to its strong **antioxidant** and **antimicrobial** activity, it is used in food preservation, especially in meat products, as well as in traditional medicine. **Clove essential oil (CEO)** shows antibacterial, antiviral, antifungal, and anticancer effects and is commonly applied for wound care, toothache relief, and various industrial uses, including perfumes, soaps, and histological preparations[15].

Figure 3:*Syzygium aromaticum* (L).

2.1.1. Table 4: Taxonomy

Table 4: Taxonomy

Kingdom	Plantae
Sub Kingdom	Tracheobionta
Super Division	Spermatophyta
Division	Magnoliphyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Genus	Syzygium
Species	Aromaticum

2.1.2. Vernacular name of *syzygiumarometicum* (L.)

Table 5: Vernacular name of *syzygium arometicum*(L.) [16]

Sanskrit	Devkusuma, Devapuspa, Lavanga
English	Clove, Clovos, Caryophyllus
Botanical	Eugenia Caryophyllus, Syzygium Aromaticum
Hindi	Lavang, Laung
Marathi	Lavang
Malayalam	Grampus, Karayampu
Kannada	Krambu, Daevakusuma, Lavanga
Tamil	Kirampu, Kiraambu, Grambu
Bengali	Lavanga
Gujrati	Lavang

2.1.3. Sources of *Syzygium aromaticum*(L.)

Biological sources:

The biological source of clove is the dried flower buds of the plant ***Syzygium aromaticum***(L.) Merrill & Perry. It belongs to the family **Myrtaceae**.

Geographical sources:

Native to Maluku Islands (Spice Islands), Indonesia

Now widely cultivated in: India (Tamil Nadu, Kerala, Karnataka),Sri Lanka, Madagascar, Tanzania (Zanzibar – major producer), Malaysia, Comoros.

Melting point :–9 °C

Boiling Point : 254 °C

Moisture Content :≤ 11%

Volatile Oil Content :15–20% volatile oil

Major Chemical Constituents: Eugenol(45–90%),Eugenyl acetate**,** B-caryophyllene**,** Tannins & flavonoids

2.1.4. Phytoconstituents present in *Syzygium aromaticum*(L.)

Phytochemicals from edible and medicinal plants play a major role in drugs for inflammation and oxidative stress. Eugenol, a key phenolic in essential oils, is widely used in medicine, dentistry, food, agriculture, and cosmetics. Considered safe by WHO, it has strong anti-inflammatory, antioxidant, analgesic, and antimicrobial properties. Clove is the richest eugenol source, with its oil containing mainly eugenol, β-caryophyllene, o-humulene, and eugenol acetate[17]. Clove contains various bioactive compounds such as phenolics, flavonoids, tannins, terpenoids, and alkaloids.

1.Phenolic compounds (Eugenol, eugenol acetate)

– Antioxidant, analgesic, antimicrobial.

2. Flavonoids

– Antioxidant & anti-inflammatory.

3. Tannins

– Astringent, antimicrobial.

4. Alkaloids

– Antimicrobial & therapeutic.

5. Terpenoids (β-caryophyllene, humulene)

– Anti-inflammatory, analgesic, antimicrobial.

2.1.5. Medicinal properties of *Syzygium aromaticum*(L.)

Clove is widely used in cosmetics, medicine, food, and agriculture because it contains rich bioactive compounds like gallic acid, flavonoids, eugenol, and eugenol acetate. Its essential oil shows strong antibacterial, antifungal, antinociceptive, and anticancer properties[18].

a) Antimicrobial activity

Clove has broad antimicrobial activity, with its eugenol-rich essential oil disrupting microbial cell walls and inhibiting pathogens like Staphylococcus aureus, E. coli, Salmonella typhi, and some viruses, including herpes and hepatitis C[19].

b) Antioxidant activity

Clove is a potent antioxidant, with phenolics and flavonoids that scavenge free radicals and reduce oxidative stress linked to aging and chronic diseases[20].

c) Anti- inflammatory activity

Clove compounds, especially eugenol, reduce inflammation by inhibiting enzymes like COX-2, easing swelling and pain in conditions such as arthritis and respiratory or digestive inflammation[21].

d) Analgesic (pain – relieving)

Clove oil, rich in eugenol, provides analgesic and anesthetic effects, commonly used to relieve toothache and gum pain[20].

e) Antidiabetic and Metabolic Effects

Cloves may help regulate blood sugar and lipid levels through antioxidant and enzyme-modulating effects, though human studies are limited[20].

f) Neuroprotective Effects

Preliminary studies indicate clove may protect neurons by reducing oxidative stress and modulating enzymes linked to neurodegeneration[22].

3.MATERIALS AND METHOD

3.1 Materials

Clove flower buds, ethanol, DMSO, Mueller–Hinton agar/broth, Staphylococcus aureus and Streptococcus pyogenes, antibiotic discs, incubator, autoclave, Preliminary test, AutoDock , and PyMOL.

3.2 Method

In Vitro Methodology

- Collection and authentication of plant material
- Preparation of clove extract
- Preliminary phytochemical screening
- Microorganisms used
- Preparation of culture media
- Antibacterial activity (Agar well diffusion method)
- Incubation
- Measurement of zone of inhibition

In Silico Methodology (Molecular Docking)

- Selection of active compound
- Ligand preparation
- Protein preparation
- Molecular docking
- Visualization of results

3.3 Collection of medicinal plants

Dried clove buds (*Syzygium aromaticum* Linn) were collected from the local market, cleaned, shade-dried, and authenticated before use.

3.4 Plant authentication

The collected plant material, dried clove buds (*Syzygium aromaticum*Linn), was authenticated by a qualified botanist/pharmacognosist through detailed examination of its morphological and taxonomical characteristics and was confirmed to belong to the family Myrtaceae before being used for the study.

3.5 Drying

Clove buds must be dried immediately after harvest to prevent fermentation. Sun drying with frequent turning ensures even browning over 4–5 days until moisture reaches 8–10%, indicated by a snapping sound; mechanical dryers are used during the rainy season.

3.6. Preparation of plant extract

Dried buds of *Syzygium aromaticum* Linn (clove) were powdered and sequentially extracted in a Soxhlet apparatus using water, methanol, ethyl acetate, and petroleum ether[23].

3.7. Soxhlet extraction

About 100g of the powdered sample was placed in a thimble and loaded into the Soxhlet apparatus. Ethanol (1:3w/v) was added to the round-bottom flask, and the system was heated on a mantle. During heating, solvent vapors condensed and percolated through the sample, dissolving the extractable components, which were periodically siphoned back into the flask. Extraction was carried out for 8 hours at 55–60 °C. After completion, heating was stopped, the solvent was evaporated, and the extracted oil was concentrated in the flask[24].The weighed extracts were stored in sealed containers for preliminary phytochemical analysis.

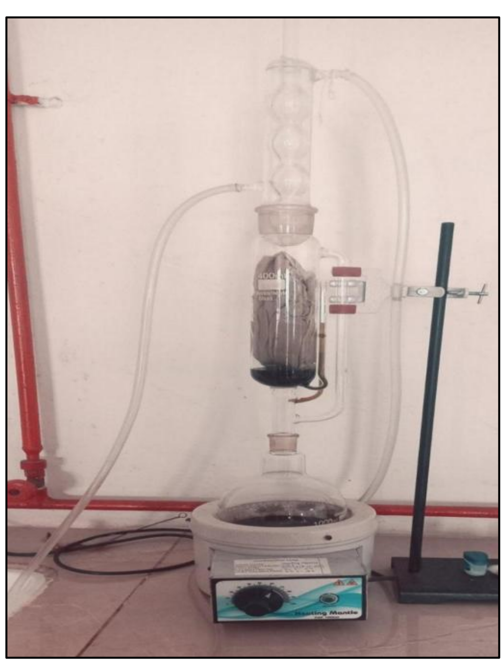

Figure 4: Soxhlet extraction

3.8. PRELIMINARY PHYTOCHEMICAL ANALYSIS

The extracts were analyzed for the presence of bioactive compounds using standard phytochemical methods.

A.Test for Carbohydrates

Molisch's test:1ml of the plant extract was mixed with 0.4 ml of Molisch's reagent, followed by the careful addition of 1 ml of concentrated sulphuric acid along the side of the test tube. Formation of a purple colour indicated the presence of carbohydrates (starch).

Benedict's test: Equal volumes (1ml each) of the plant extract and Benedict's reagent were heated for 5 minutes. Formation of an orange precipitate indicated the presence of carbohydrates (disaccharides).

Fehling's test: 1ml of plant extract was boiled with 2 ml of purified water and filtered. Two millilitres of Fehling's reagent were added to 2 ml of the filtrate and heated. Formation of a reddish-brown precipitate indicated the presence of carbohydrates (glucose).

B. Test for proteins:

Xanthoproteic test: To 1 ml of the plant extract, 0.25 ml of nitric acid was added. Formation of a white precipitate indicated the presence of proteins.

Biuret test: 1ml of plant extract was treated with 4% NaOH and 1% $CuSO_4$. Development of a violet-pink colour confirmed the presence of proteins.

C. Test for lipids:

Solubility test: 1ml of plant extract was evaporated to dryness, treated with a few drops of petroleum ether, and shaken. Complete dissolution indicated the presence of lipids.

Glycerol test: Five drops of the plant extract were added to 1 ml of 1% $CuSO_4 \cdot 5H_2O$, followed by five drops of 10% NaOH. Formation of a clear blue solution indicated the presence of glycerol.

Sudan III test: A few drops of Sudan III solution were added to 1 ml of the plant extract. Development of a red colour confirmed the presence of lipids.

D. Test for alkaloids:

Mayer's test: 1ml of clove flower bud extract was treated with two drops of chloroform and Mayer's reagent. Formation of white deposits indicated the presence of alkaloids.

Wagner's test:1ml of the extract was treated with Wagner's reagent. Appearance of a brown-reddish precipitate confirmed alkaloids.

Dragendorff's test:2ml of Dragendorff's reagent were added to 1 ml of the plant extract. Formation of an orange-white precipitate indicated the presence of alkaloids.

E. Test for tannins:

Gelatin test: To 500 µl of the filtrate, 1% gelatin solution was added. Formation of a curdy white precipitate indicated tannins.

Lead acetate test: Addition of 5 ml of 10% lead acetate to the filtrate produced a white precipitate, confirming tannins.

Ferric chloride test: Five drops of 5% ferric chloride were added to the filtrate. Development of a blue-green colour indicated tannins.

F. Test for saponins:

Foam test: 1ml of plant extract was mixed with a little water and sodium bicarbonate, then shaken vigorously for 5 minutes. Formation of foam indicated the presence of saponins.

G. Flavonoids test: To 0.5 ml of plant extract, 5 ml dilute ammonia and 1 ml concentrated sulphuric acid were added. A yellow colour that fades on standing indicates flavonoids.

H. Resins test: To 0.5 ml acetic acid in a dry tube, 2 drops of conc. H_2SO_4 were added. Purple colour turning violet in ~10 minutes indicates resins**.**

I. Sterols test (Salkowski): To 0.5 ml extract, 2 ml chloroform and 2 ml conc. H_2SO_4 were added along the tube wall and gently shaken. Red colour in the chloroform layer indicates sterols.

J.Keller–Killiani test: The plant extract was evaporated at 40 °C, and the residue obtained was dissolved in water. To this, glacial acetic acid containing a drop of ferric chloride was added, followed by careful underlaying with concentrated sulphuric acid. Formation of a brown ring at the interface indicated the presence of deoxy sugars characteristic of cardiac glycosides.

K.Test for Anthraquinones:2 ml of plant extract was shaken with 10 ml benzene, then 5 ml of 10% ammonia was added and mixed. A pink color in the ammonical layer indicates the presence of anthraquinones[23].

3.9. INVITRO ANTIBACTERIAL ACTIVITY

3.9.1. Cultivation of bacteria

The isolation and cultivation of bacteria from environmental or animal-derived material is a foundational practice in microbiology Staphylococcus aureus can be obtained from mouse feces by Homogenizing the samples in a suitable buffer and inoculating them onto selective media, such as Mannitol Salt Agar, followed by incubation at 37 °C for 24-48 hours[25].

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Figure 5: Cultivation of bacteria

3.9.2. Bacterial Identification and Characterization

Procedure

Gram staining is a differential technique used to classify bacteria based on cell wall composition, aiding in identification and guiding diagnostic or treatment decisions.

Preparation of a slide smear

1. Use an inoculation loop to transfer a small amount of culture onto a microscope slide.
2. Add a drop of water if using colonies from a Petri dish or slant to facilitate spreading.
3. Spread the culture into a thin, even film about 15 mm in diameter; multiple smears (up to 4) can fit on one slide.
4. Air-dry the smear or gently heat it over a flame, moving the slide in a circular motion to avoid overheating.
5. Heat fixation ensures cell adhesion and prevents loss of material during staining.

Gram staining

Primary Stain: Cover the fixed smear with crystal violet for 10–60 seconds, then rinse gently with water.

Mordant: Apply iodine solution for 10–60 seconds to fix the dye, then rinse with water.

Decolorization: Add a few drops of ethanol-acetone decolorizer briefly, then rinse with water; stop once the solvent runs clear.

Counterstain: Apply basic fuchsin for 40–60 seconds, rinse with water, and blot dry or air-dry.

Microscopic Examination of Slide

- Examine the slide under a microscope using oil immersion.
- Start with the 40× objective to assess smear distribution, then use the 100× oil immersion objective for detailed observation.
- Focus on areas where cells are one layer thick; avoid thick areas to prevent inaccurate results.
- Note that white blood cells and macrophages appear Gram-negative, while squamous epithelial cells appear Gram-positive.

Microorganisms are identified by their shape and Gram reaction: Gram-positive cells stain purple or blue, Gram-negative cells stain pink or red; bacilli are rod-shaped, while cocci are spherical[26].

Motility of staphylococcus aureus

Staphylococcus aureus has long been considered a non-motile bacterium because it lacks flagella. However, recent studies have demonstrated that S. aureus is capable of surface-associated movement on soft agar. This movement occurs through two distinct mechanisms: spreading, where bacterial colonies expand outward as a thin layer over the agar surface, and comet formation, in which groups of cells move directionally, producing tail-like trails resembling comets. These motility-like behaviors are thought to be driven by cell growth, surfactant production, and interactions with the agar surface, rather than true flagellar motility[27].

Procedure

Slide preparation: Apply a thin ring of petroleum jelly around the concave well of a cavity slide.

Inoculation: Place a single loopful of 24-hour Staphylococcus aureus broth culture at the center of a clean, grease-free coverslip.

Assembly: Gently place the cavity slide (depression facing downward) over the coverslip, ensuring the drop aligns with the cavity; seal using petroleum jelly.

Inversion: Carefully invert the slide so the culture drop hangs freely into the cavity.

Microscopic observation:

- Place the slide on the microscope stage.
- Locate the edge of the hanging drop under 10× objective.
- Reduce light intensity using the iris diaphragm to improve contrast.
- Examine motility under the 40× high-dry objective.

Result: Staphylococcus aureus appears as non-motile cocci, exhibiting only Brownian movement (vibration in place) due to the absence of flagella.

3.9.3.Antibacterial activity of plants extract

The antibacterial activity of plant extracts was evaluated against Staphylococcus aureus at different concentrations using the agar well diffusion method. Antimicrobial efficacy was determined by measuring the zones of inhibition, with clove extract used as the test sample. All procedures were carried out under strict aseptic conditions to prevent contamination. Laboratory glassware, including beakers, volumetric flasks, droppers, measuring cylinders, pipettes, conical flasks, and glass bottles, was sterilized by autoclaving at 121 °C for 20 minutes before use[28].

3.9.4. Preparation of Nutrient Agar

Nutrient agar was completely dissolved in distilled water and sterilized by autoclaving at 120 °C under 15–20 lbs pressure for 30 minutes. The sterilized nutrient agar medium was then poured into sterile Petri plates under laminar airflow conditions in an undisturbed environment to avoid contamination. The medium was allowed to spread uniformly over the plates. Subsequently, the agar plates were incubated at 37 °C overnight to allow proper solidification of the nutrient agar[29].Once the nutrient agar has solidified, the plates are aseptically inoculated with Staphylococcus aureus culture by spread plate or streak plate method and incubated at 37 °C for 24 hours to allow bacterial growth.

3.9.5. Screening of antibacterial activity of the Plant extracts

The antibacterial activity was evaluated using the agar well diffusion method. The agar surface was uniformly inoculated with the reference bacterial strain using a sterile cotton swab, and wells were aseptically prepared in the agar using a sterile cork borer[28].

In this method, clove oil was used as the test sample, while ethanol served as the control, as it is an effective solvent for essential oils. Different concentrations of clove oil were prepared by mixing 0.1 mL, 0.25 mL, 0.5 mL, 1.0 mL, 1.5 mL, and 2.0 mL of clove oil with 9.9 mL, 9.75 mL, 9.5 mL, 9.0 mL, 8.5 mL, and 8.0 mL of ethanol, respectively, to obtain a final volume of 10 mL for each concentration.The plates were allowed to stand for 30 min and then incubated aerobically at 37 °C for 48 h.The zones of inhibition around the wells were measured. The mean zone diameter was recorded[28].

3.9.6. Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined using the agar well diffusion method[30].The minimum Inhibitory concentration (MIC) was defined as the lowest concentration of the antibacterial agent that inhibited visible bacterial growth.Mueller–Hinton agar plates were uniformly inoculated with the test bacterial suspension (approximately 5×10^5 CFU/mL) using a sterile cotton swab. Wells were aseptically punched using a sterile cork borer, and different volumes of clove extract (0.1, 0.25, 0.5, 1.0, 1.5, and 2.0 mL) were added into the respective wells. Ethanol served as the negative control, while wells without extract served as the growth control. The plates were Incubated at 37 °C for 24 h, and antibacterial activity was evaluated based on the presence or absence of bacterial growth and the diameter of the zone of inhibition. The lowest volume of clove extract that produced a clear zone of inhibition with no visible growth was considered the MIC[31].

3.9.7. Minimum bactericidal concentration

The minimum bactericidal concentration (MBC) was determined by subculturing samples from the inhibition zones onto nutrient agar plates. The lowest volume of the extract that showed no bacterial growth upon subculture was considered the MBC for the test organism[31].

3.10. IN SILICO

1.Retrieval of Protein Structure

The three-dimensional (3D) structure of the target protein implicated in impetigo skin infection was retrieved from the Protein Data Bank (PDB ID: IVQQ). The protein structure was downloaded in PDB format. Prior to docking, the protein was prepared by removing water molecules, adding polar hydrogen atoms, and assigning Kollman charges to stabilize the structure.

2.Retrieval and Preparation of Ligand

The phytochemical compound eugenol, the major bioactive constituent of *Syzygiumaromaticum*Linn (clove), was selected as the ligand for the study. The 3D structure of eugenol was obtained from the PubChem database (PubChem Compound ID: 3314; Molecular formula: C10H12O2). The ligand structure was energy minimized and converted into PDBQT format for docking analysis.

3.Active Site Identification

The active binding site of the target protein was identified based on the co-crystallized ligand region and literature studies. Grid box parameters were set around the predicted active site residues to ensure proper ligand binding during docking simulation.

4.Molecular Docking Study

Molecular docking analysis was carried out using AutoDock. The prepared protein and ligand files were converted into PDBQT format using AutoDock tools. The grid box was defined to cover the active site region of the protein. Docking was performed using the Lamarckian Genetic Algorithm to predict the best possible binding conformations. Binding affinity was evaluated based on binding energy (kcal/mol), hydrogen bond interactions, and hydrophobic interactions between eugenol and the target protein.

5.Visualization and Analysis

The docked protein-ligand complex was visualized and analyzed using molecular visualization software such as PyMOL to study the interaction pattern, hydrogen bonds, and binding orientation of eugenol within the active site of the protein[32].

4. RESULTS AND DISCUSSION

4.1. Percentage yield of clove oil

Clove oil was extracted from dried clove buds using the Soxhlet extraction method. After completion of extraction and solvent evaporation, 12 mL of clove oil was obtained. The extracted oil was pale yellow in colour with a characteristic aromatic odour.

Percentage yield was calculated using the following formula:

$$\text{Percentage yield} = (\text{Amount of extract obtained} / \text{Weight of plant material used}) \times 100$$

$$\text{Percentage yield} = (12\ \text{mL} / 100\ \text{g}) \times 100 = 12\ \%\ (\text{v/w})$$

The percentage yield obtained indicates that Soxhlet extraction is an efficient method for the recovery of essential oil from clove buds.

Figure 6: Clove oil

4.2 Phytochemical screening

Preliminary phytochemical screening of the clove (*Syzygiumaromaticum* Linn) extract was carried out using selected qualitative chemical tests. The results were interpreted based on the visible colour changes observed in the reaction mixtures (Figure7).

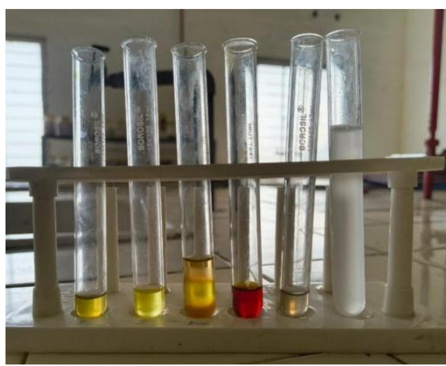

Figure 7: Phytochemical screening of clove oil

The extract showed positive reactions for phenolic compounds, flavonoids, glycosides, and carbohydrates, as indicated by the development of characteristic yellow, brown, and reddish coloration. No precipitate or colour change indicative of proteins or amino acids was observed in the tests performed.

Table 6: Phytochemical screening of clove oil

S.No	Phytochemical	Test Performed	Observation	Inference
1.	Carbohydrates	Molisch's test	Yellowish ring / colour formation	Present (+)
2.	Glycosides	Keller–Killiani test	Brown ring at the interface	Present (+)
3.	Flavonoids	Alkaline reagent test	Yellow coloration	Present (+)
4.	Phenols	Ferric chloride test	Dark yellow to brown coloration	Present (+)
5.	Proteins	Biuret test	No violet colour	Absent (–)
6	Amino acids	Ninhydrin test	No purple colour	Absent (–)

4.3 INVITRO STUDY

4.3.1. Microscopic Analysis of Staphylococcus aureus Using Gram Staining

Microscopic observation of the stained smear revealed purple-colored, spherical bacterial cells arranged predominantly in grape-like clusters. The organisms retained the crystal violet stain, indicating a Gram-positive reaction. The observed cellular morphology and staining characteristics are consistent with Gram-positive cocci of Staphylococcus aureus. These findings are summarized in Table 7, and a representative micrograph is shown in Figure 8.

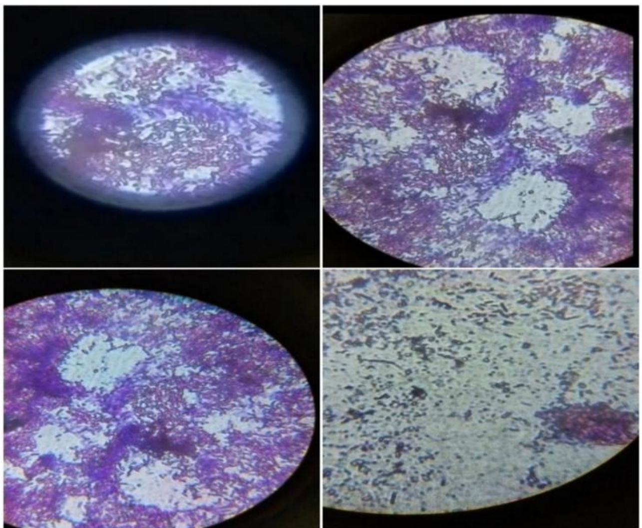

Figure 8: Gram-stained micrograph of Staphylococcus aureus showing purple cocci arranged in grape-like clusters (100× oil immersion)

Table 7:Observations of Staphylococcus aureus under Gram staining

Parameters	Observation	Inference / Remarks
Staining Method	Gram staining	Differential staining
Color of Cells	Purple	Gram-positive
Cell Shape	Spherical (cocci)	Typical morphology of S. aureus
Arrangement	Irregular clusters (grape-like)	Characteristic of S. aureus
Magnification	100× oil immersion	Standard for bacterial morphology
Motility	Non-motile (from literature)	Confirms S. aureus identity
Notes	No contamination observed	Smear prepared under sterile conditions

4.3.2. Screening of antibacterial activity of clove oil against Staphylococcus aureus

The antibacterial activity of clove oil was screened using the agar well diffusion method against Staphylococcus aureus. Different concentrations of clove oil (0.1, 0.25, 0.5, 1.0, 1.5, and 2.0 mL) were tested, with ethanol serving as the negative control.

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At lower concentrations (0.1 and 0.25 mL), no clear zone of inhibition was observed, indicating minimal or no antibacterial activity. A small but distinct zone of inhibition appeared at 0.5 mL concentration. As the concentration increased to 1.0, 1.5, and 2.0 mL, a progressive increase in the diameter of the inhibition zone was observed, demonstrating a concentration-dependent antibacterial effect. The maximum zone of inhibition was recorded at 2.0 mL of clove oil.

The ethanol control did not exhibit any zone of inhibition, confirming that the antibacterial activity observed was due to the clove oil extract and not the solvent.

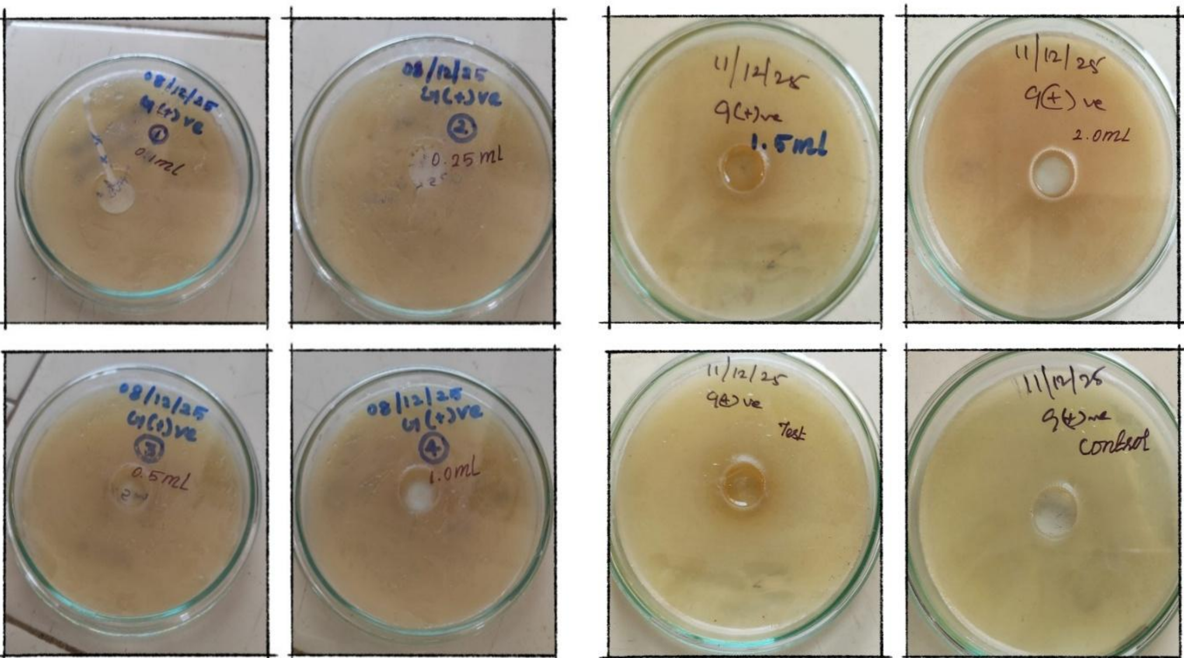

Figure 9 : Screening of antibacterial activity of clove oil against Staphylococcus aureus by agar well diffusion method at different concentrations.

Discussion

The present study demonstrates that clove oil exhibits significant antibacterial activity against Staphylococcus aureus, as indicated by clear zones of inhibition in the agar well diffusion assay. The activity was concentration dependent, with no inhibition observed at lower concentrations (0.1 and 0.25 mL), moderate inhibition at 0.5 mL, and pronounced inhibition at higher concentrations (1.0–2.0 mL). This antibacterial effect is mainly attributed to eugenol, a phenolic compound known to disrupt bacterial cell membranes and cause leakage of intracellular components. The absence of inhibition in the ethanol control confirms that the activity was due to clove oil alone. These findings support previous reports on the efficacy of clove oil against Gram-positive bacteria and suggest its potential as a natural antibacterial agent against Staphylococcus aureus.

4.3.3. Zone of inhibition of clove oil against Staphylococcus aureus

The antibacterial activity of clove oil was evaluated against Staphylococcus aureus using the agar well diffusion method. The zone of inhibition produced by different concentrations of clove oil is presented in Table 8. A concentration-dependent increase in the zone of inhibition was observed. No inhibition was noted at lower concentrations (0.1 and 0.25 mL), whereas higher concentrations produced measurable and progressively larger inhibition zones.

Table 8: Zone of inhibition of clove oil against Staphylococcus aureus

Clove oil concentration (mL/10 mL)	Zone of inhibition(mm)	Activity
0.1	-	No inhibition
0.25	8 ± 0.5	Low
0.5	11 ± 0.6	Moderate
1.0	15 ± 0.7	Good
1.5	18 ± 0.5	Very good
2.0	21 ± 0.8	Excellent
Ethanol (control)	-	No inhibition

Values are expressed as mean ± SD of triplicate experiments; (–) indicates no inhibition.

A graphical representation of the zone of inhibition at different concentrations of clove oil is shown in Figure 10.

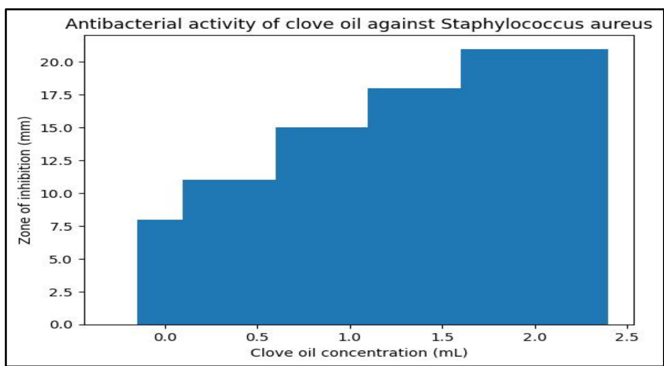

Figure 10: Zone of inhibition of clove oil against Staphylococcus aureus at different concentrations.

4.3.4. Determination of minimum inhibitory concentration

Materials Required

- Clove oil (commercial or laboratory extracted)
- Test organism (24-hour bacterial culture)
- Nutrient agar / Mueller–Hinton agar
- Nutrient broth (for culture preparation only)
- Dimethyl sulfoxide (DMSO) or ethanol (solvent)
- Sterile Petri plates
- Sterile cork borer / well puncher
- Micropipettes with sterile tips
- Sterile cotton swabs
- Incubator (37 °C)
- McFarland standard (0.5)

- Sterile saline
- Vernier caliper / ruler (for zone measurement)
- Control antibiotic discs or solution (optional)

1. Preparation of Clove Oil Stock Solution

Dissolve clove oil in DMSO/ethanol

Example stock: 10 mg/mL

2.Agar well diffusion method

Table 9: Concentrations of Clove Oil Used in Agar Well Diffusion Assay

Well No.	Concentration of Clove Oil (mg/mL)
W1	0.1
W2	0.25
W3	0.5
W4	1.0
W5	1.5
W6	2.0

3. Inoculum Preparation

- Adjust bacterial suspension to 0.5 McFarland standard
- (~1.5 x 108 CFU/mL)
- Dilute to working concentration (~10° CFU/mL)

4.Inoculation

- Add 100 µL bacterial suspension to each tube/well
- Add 100 µL clove oil dilution
- Final volume = 200 µL per well

5.Controls

- Positive control → bacteria + broth
- Negative control → broth only
- Solvent control → bacteria + solvent

6.Incubation

- Incubate at 37°C for 18-24 hours

7.Observation

- After incubation, agar plates were observed for clear zones of inhibition around the wells.

- The diameter of each inhibition zone was measured in millimeters (mm) using a ruler or Vernier calliper.
- Larger zones of inhibition indicated higher antibacterial activity of clove oil.
- Absence of a clear zone indicated no antibacterial effect.
- Results were compared with solvent control and standard antibiotic control.

MIC BROTH DILUTION SETUP

Table10: MIC broth dilution setup

2mg/ml	1.5mg/ml	1mg/ml	0.5mg/ml	0.25mg/ml	0.1mg/ml	Control
+	+	+	+	+	+	+

Bacteria added to each well/tube

Clear wells = inhibition

Turbid wells = growth

MIC = first clear well

Result

The MIC of clove oil against the test organism was found to be 0.5 mg/mL, which represents the lowest concentration that completely inhibited visible microbial growth after 24 hours of incubation at 37°C.

Table 11: Determination of MIC of the test sample against the test organism

Concentration (mg/ml)	Growth
2.0	No growth
1.5	No growth
1.0	No growth
0.5	No growth
0.25	Slight growth
0.1	Growth
Control	Growth

4.3.5. MIC Determination Curve

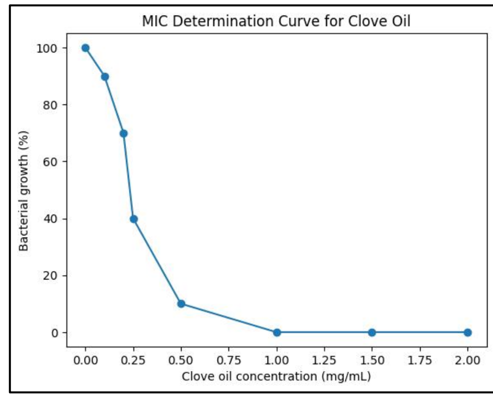

Figure 11: MIC Determination Curve of Clove Oil Against Staphylococcus aureus

MIC Value:

MIC = 0.5 mg/mL

(lowest concentration with no visible growth)

Discussion

Clove oil shows strong antimicrobial activity due to eugenol. The MIC value confirms its dose-dependent inhibitory effect on microbial growth.

4.3.6. Minimum bactericidal concentration

Materials Required

- Clove oil
- Test organism (24-hour culture)
- Mueller-Hinton broth
- Mueller-Hinton agar plates
- DMSO / ethanol (solvent)
- Sterile Petri plates
- Sterile micropipettes and tips
- Sterile cotton swabs
- Incubator (37 °C)

1.Preparation of Agar Plate

- Prepare Mueller–Hinton agar and pour into sterile Petri plates.
- Allow the agar to solidify at room temperature.

2.Inoculation of Test Organism

- Take a 24-hour bacterial culture.
- Swab uniformly over the agar surface to form a bacterial lawn.

3.Formation of Agar Wells

- Make wells (≈6 mm diameter) in the agar using a sterile cork borer.

4.Addition Oil Concentrations

- Add different concentrations of clove oil into separate wells.
- 0.1 ml

- 0.25 ml
- 0.5 ml
- 1.0 ml
- 1.5 ml
- 2.0 ml
- Use DMSO/ethanol as solvent control.

5.Diffusion and Incubation

- Allow plates to stand for 30 minutes for diffusion.
- Incubate at 37 °C for 24 hours.

6.Observation

- Observe plates for zones of inhibition.
- Check for complete absence of bacterial growth around wells.

Result

The MBC of clove oil against the test bacterium was found to be 1.0 mg/mL, indicating the lowest concentration that completely killed the bacterial cells.

Table 12: Determination of Minimum Bactericidal Concentration (MBC) of Clove Oil by Agar Well Diffusion Method

Concentration (mg/mL)	Colonies observed	Result
0.1	Heavy bacterial growth	Not bactericidal
0.25	Many colonies	Not bactericidal
0.5	Few colonies	Not bactericidal
1.0	No bacterial colonies Bactericidal	Bactericidal
1.5	No bacterial colonies Bactericidal	Bactericidal
2.0	No bacterial colonies Bactericidal	Bactericidal

4.3.7. MBC Determination Curve

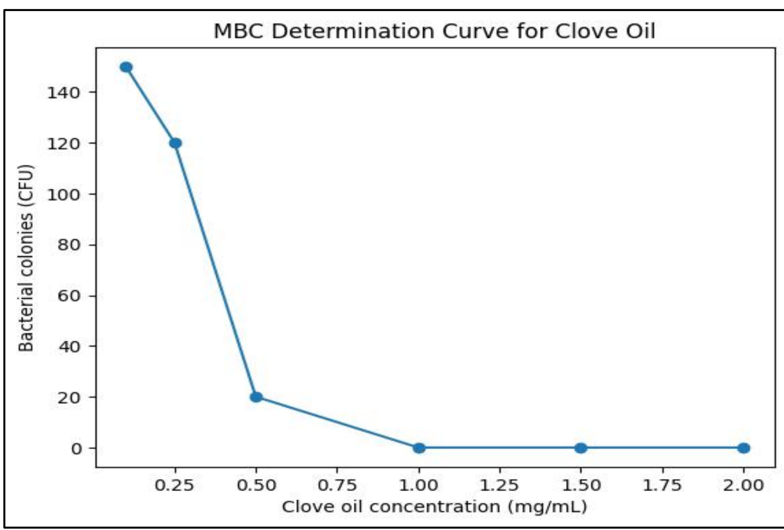

Figure 12: MBC Determination Curve of Clove Oil Against Staphylococcus aureus

MBC Value

MBC = 1.0 mg/mL

(Lowest concentration showing complete absence of bacterial colonies)

4.4 IN SILICO

1.Target Protein Information

The three-dimensional structure of the target protein (PDB ID: IVQQ) was retrieved from the Protein Data Bank. The protein corresponds to dehydrosqualene synthase of Staphylococcus aureus, a key enzyme involved in bacterial virulence.

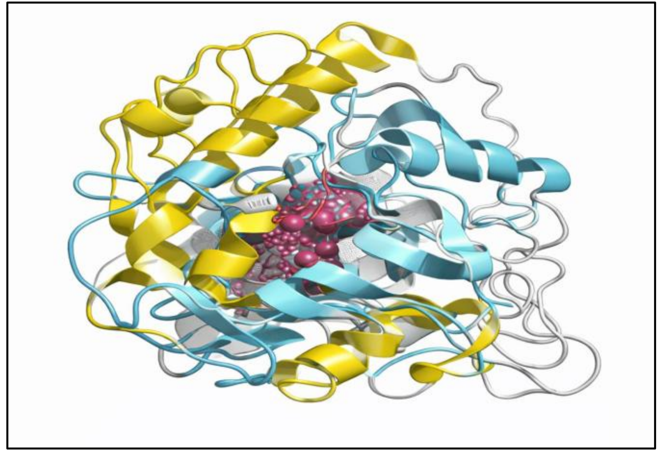

Figure 13: 3D Structure of Target Protein (IVQQ)

2. Ligand Information

The selected ligand was Eugenol, the major bioactive compound of *Syzygium aromaticum* Linn (clove).

The 3D structure was retrieved from PubChem (CID: 3314).

Table 13: Physicochemical Properties of Eugenol

Property	Value
Ligand Name	Eugenol
Molecular Formula	C10H12O2
Molecular Weight	164.20 g/mol
PubChem ID	3314
Chemical Class	Phenolic compound

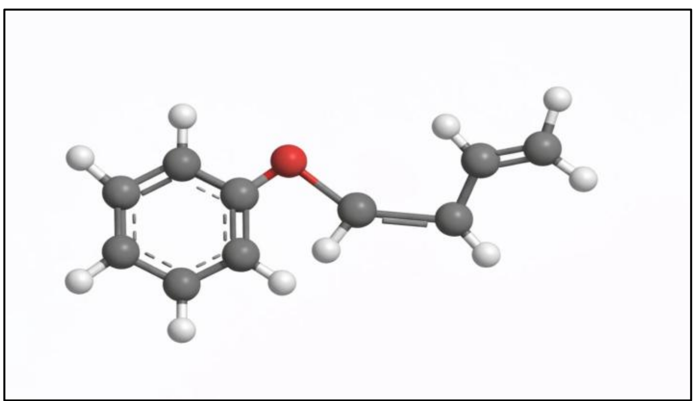

Figure 14: 3D Structure of Eugenol

3. Docking Outcome

Molecular docking was performed using AutoDock with the Lamarckian Genetic Algorithm.

Binding Energy –5.9 kcal/mol

The negative binding energy indicates favorable interaction between eugenol and IVQQ protein.

2. Interaction Analysis

Table 14: Docking Interaction Summary

Parameter	Observation
Binding Energy	–5.9 kcal/mol
Hydrogen Bonds	1
Hydrophobic Interactions	Present
Binding Stability	Moderate

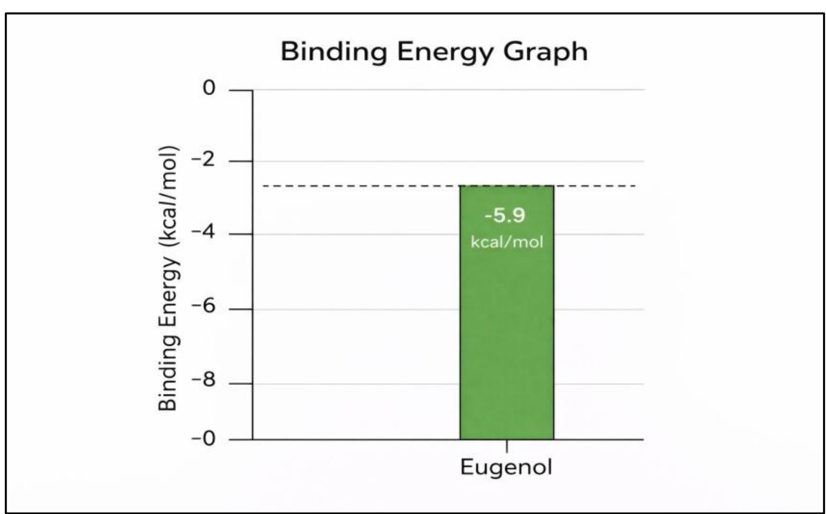

Figure 15: Binding Energy Graph

Discussion

The molecular docking study showed that eugenol interacts favorably with the IVQQ protein (dehydrosqualene synthase of Staphylococcus aureus). The binding energy was found to be –5.9 kcal/mol, which indicates a stable and moderate interaction between the ligand and the protein. The formation of one hydrogen bond contributes to the proper positioning and stability of eugenol within the active site. In addition, hydrophobic interactions were observed, which further enhance the stability of the protein–ligand complex. Since this enzyme plays an important role in bacterial virulence, its interaction with eugenol suggests that the compound may help inhibit bacterial activity. Overall, the results support the potential antibacterial property of eugenol, but further experimental studies are necessary to confirm its effectiveness.

5. SUMMARY AND CONCLUSION

5.1. Summary

The present study aimed to evaluate the **In silico and In vitro antibacterial activity of *Syzygium aromaticum*Linn (clove)** against bacterial pathogens implicated in impetigo skin disease, with particular emphasis on Staphylococcus aureus. Clove oil was extracted using the Soxhlet extraction method and subjected to preliminary phytochemical screening, which confirmed the presence of bioactive constituents such as phenolics, flavonoids, alkaloids, and glycosides. These phytochemicals are known to contribute to antimicrobial activity.

The **In vitro antibacterial potential** of clove oil was assessed using the agar well diffusion method at different concentrations. The results demonstrated a concentration-dependent increase in the zone of inhibition, indicating effective antibacterial activity against the test organism. The minimum inhibitory concentration (MIC) was identified at lower concentrations, highlighting the potency of clove oil even at minimal doses.

In the **In silico analysis,** molecular docking studies were performed to investigate the interaction between major bioactive compounds of clove (such as eugenol) and selected target proteins of Staphylococcus aureus. The docking results revealed strong binding affinity and favorable interactions, suggesting inhibition of essential bacterial functions. The in silico findings supported the in vitro results, indicating a strong correlation between computational predictions and laboratory observations.

Overall, the study provides scientific evidence supporting the antibacterial efficacy of *Syzygium aromaticum*(L.) against impetigo-associated bacteria through both experimental and computational approaches.

5.2 Conclusion

The findings of the present study conclude that ***Syzygium aromaticum*** **(L.)possesses significant antibacterial activity** against bacteria implicated in impetigo skin disease. The in vitro results demonstrated a clear, concentration-dependent inhibition of

bacterial growth, confirming the effectiveness of clove oil as a natural antibacterial agent. The presence of bioactive phytochemicals, particularly phenolic compounds such as eugenol, plays a crucial role in this activity.

Furthermore, the in silico molecular docking studies revealed strong interactions between clove bioactive compounds and bacterial target proteins, supporting the proposed mechanism of antibacterial action. The agreement between in silico and in vitro findings strengthens the reliability of the results and highlights the potential of clove as a source of alternative therapeutic agents.

In conclusion, *Syzygium aromaticum*(L.) shows promising potential as a **natural, cost-effective, and safe antibacterial agent** for the management of impetigo-causing infections. However, further studies involving advanced characterization, toxicity analysis, and in vivo evaluations are recommended to validate its clinical applicability and formulation into topical antimicrobial therapies.

6. REFERENCES

1.Lotfollahi Z. The anatomy, physiology and function of all skin layers and the impact of ageing on the skin. Wound Practice & Research: Journal of the Australian Wound Management Association. 2024 Mar;32(1):6-10.
2.Lim KM. Skin epidermis and barrier function. International journal of molecular sciences. 2021 Mar 16;22(6):3035.
3.Yousef H, Alhajj M, Sharma S. Anatomy, skin (integument), epidermis.
4.Anderson BJ, Wilz L, Peterson A. The identification and treatment of common skin infections. Journal of athletic training. 2023 Jun 1;58(6):502-10.
5.Ki V, Rotstein C. Bacterial skin and soft tissue infections in adults: a review of their epidemiology, pathogenesis, diagnosis, treatment and site of care. Canadian Journal of Infectious Diseases and Medical Microbiology. 2008;19(2):173-84.
6.Hartman-Adams H, Banvard C, Juckett G. Impetigo: diagnosis and treatment. American family physician. 2014 Aug 15;90(4):229-35.
7.Hoffmann TC, Peiris R, Glasziou P, Cleo G, Del Mar C. Natural history of non-bullous impetigo: a systematic review of time to resolution or improvement without antibiotic treatment. British Journal of General Practice. 2020 Dec 9.
8.Jeffrey W. Scales, MD; Alan B. Fleischer Jr, MD. Bullous Impetigo. Arch Pediatr Adolesc Med Published Online: November 1997. 1997;151;(11):1168-1169.
9.Nardi NM, Schaefer TJ. Impetigo.[Updated 2023 Jul 31]. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. 2024.
10.Schachner LA, Lynde CW, Kircik LH, Torrelo A, Hohl D, Kwong P, Oza V, Andriessen A, Hebert AA. Treatment of Impetigo and Antimicrobial Resistance. Journal of Drugs in Dermatology: JDD. 2021 Apr 1;20(4):366-72.
11.Toschi A, Giannella M, Viale P. Recurrence of skin and soft tissue infections: identifying risk factors and treatment strategies. Current opinion in infectious diseases. 2025 Apr 1;38(2):71-7.
12.Koning S, van der Sande R, Verhagen AP, van Suijlekom-Smit LW, Morris AD, Butler CC, Berger M, van der Wouden JC. Interventions for impetigo. Cochrane Database of Systematic Reviews. 2012(1).
13.Alanazi AK, Alqasmi MH, Alrouji M, Kuriri FA, Almuhanna Y, Joseph B, Asad M. Antibacterial activity of Syzygium aromaticum (clove) bud oil and its interaction with imipenem in controlling wound infections in rats caused by methicillin-resistant Staphylococcus aureus. Molecules. 2022 Dec 5;27(23):8551.
14.Tariq H, Alhudhaibi AM, Abdallah EM. Syzygium aromaticum (clove buds) as a natural antibacterial agent: a promising alternative to combat multidrug-resistant bacteria. Frontiers in microbiology. 2025 Oct 14;16:1674590.
15.Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. Syzygium aromaticum L.(Myrtaceae): Traditional uses, bioactive chemical constituents, pharmacological and toxicological activities. Biomolecules. 2020 Jan 30;10(2):202.
16.Yadav S, Gupta SK, Bharti D, Yogi B. Syzygium aromaticum (clove): a review on various phytochemicals and pharmacological activities in medicinal plant. World J Pharm Res. 2020 Jul 28;9:349-63.
17. R Hema Krishna. Studies on the phytochemicals of clove and their biological activities. International Journal of Advanced Chemistry. 2024 Jan 31;12(1):35-46.
18.Pandey VK, Srivastava S, Dash KK, Singh R, Dar AH, Singh T, Farooqui A, Shaikh AM, Kovacs B. Bioactive properties of clove (Syzygium aromaticum) essential oil nanoemulsion: A comprehensive review. Heliyon. 2024 Jan 15;10(1).
19. Verma A, Toshi N, Mungi V, Mehta A. The medicinal properties of clove with special focus on antimicrobial effect: a systematic review. Journal of Medical Science. 2024 Dec 31;93(4):e1156-e1156.
20.Abdelmuhsin AA, Sulieman AM, Salih ZA, Al-Azmi M, Alanaizi NA, Goniem AE, Alam MJ. Clove (Syzygium aromaticum) pods: revealing their antioxidant potential via GC-MS analysis and computational insights. Pharmaceuticals. 2025 Mar 31;18(4):504.
21.Abdul Aziz AH, Rizkiyah DN, Qomariyah L, Irianto I, Che Yunus MA, Putra NR. Unlocking the full potential of clove (Syzygium aromaticum) spice: an overview of extraction techniques, bioactivity, and future opportunities in the food and beverage industry. Processes. 2023 Aug 15;11(8):2453.

22.Sargsyan T, Simonyan HM, Stepanyan L, Tsaturyan A, Vicidomini C, Pastore R, Guerra G, Roviello GN. Neuroprotective properties of clove (Syzygium aromaticum): state of the art and future pharmaceutical applications for Alzheimer's disease. Biomolecules. 2025 Mar 20;15(3):452.

23.Lone ZA, Jain NK. Phytochemical Analysis of Clove (Syzygium aromaticum) Dried Flower Buds Extract and its Therapeutic Importance. Journal of Drug Delivery and Therapeutic. 2022;12(4-S):87-92.

24.Adegbanke OR, Bada RT. Comparative analysis of oil extraction from clove and ginger using maceration and soxhlet methods: Physicochemical properties and quality assessment. Journal of Agriculture, Food Technology and Sustainability. 2024 Aug 16;1(1):10-9.

25.Bhalla A, Aron DC, Donskey CJ. Staphylococcus aureus intestinal colonization is associated with increased frequency of S. aureus on skin of hospitalized patients. BMC infectious diseases. 2007 Sep 11;7(1):105.

26.Tripathi N, Zubair M, Sapra A. Gram staining. InStatPearls [Internet] 2025 Mar 28. StatPearls Publishing.

27.Pollitt EJ, Diggle SP. Defining motility in the Staphylococci. Cellular and Molecular Life Sciences. 2017 Aug;74(16):2943-58.

28.Yelmate AA, Dr.Thonte SS. Antibacterial activity of some Indian medicinal plants against methicillin resistant Staphylococcus aureus (MRSA). Journal of Pharmacognosy and Phytochemistry. 2019;8(5):376-380.

29.Tamta G, Mehra N, Tandon S, Nand V, Biswas S. In vitro antimicrobial assay of leaves, bark and fruits of Ficus auriculata collected from two different regions of Uttarakhand. Int J Chem Stud. 2021;9(1):930-5.

30.Pandey A, Devkota A, Yadegari Z, Dumenyo K, Taheri A. Antibacterial properties of citric acid/β-alanine carbon dots against gram-negative bacteria. Nanomaterials. 2021 Aug 6;11(8):2012.

31.Sameer S Faujdar. Antibacterial activity of Syzygium aromaticum (clove) against Uropathogens producing ESBL, MBL, and AmpC beta-lactamase: Are we close to getting a new antibacterial agent?. J Family Med Prim Care. 2020 Jan 28;9(1):180-186.

32.Kumar RS, Navatheesh S. In silico screening of lead molecule from medicinal plants for bacterial skin disease-impetigo. Research Journal of Pharmacy and Technology. 2017 Nov 1;10(11):3687-91.

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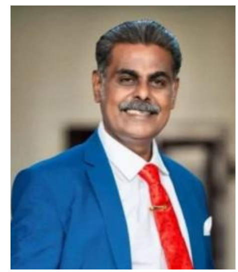	**Dr. D.Benitojohnson** **Professor and HOD.** Department of Pharmacology, RVS College of Pharmaceutical Sciences, Coimbatore, Tamil Nadu, Affiliated to The Tamil Nadu Dr MGR Medical University, Guindy, Chennai - 600032, Tamil Nadu, India.
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