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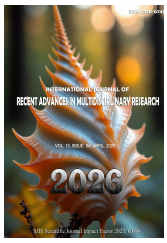

RESEARCH ARTICLE

DOSE DEPENDENT PHARMACOLOGICAL ACTIVITY OF EUCALYPTUS GLOBULUS ESSENTIAL OIL AGAINST BIOFILM FORMING MULTIDRUG RESISTANT BACTERIA

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ABSTRACT

The medicinal efficacy and low adverse effects of herbal remedies make them popular. Due to the presence of bioactive substances as 1,8-cineole, flavonoids, terpenoids, tannins, and phenolic compounds, Eucalyptus globulus is a significant medicinal plant recognized for its pharmacological activity. Using both in vitro and in vivo experimental settings, the current work attempts to assess the dose-dependent antibacterial and anti-biofilm activities of Eucalyptus globulus essential oil. Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus were among the bacteria against which antimicrobial activity was assessed using the agar well diffusion technique. The microtiter plate test was used to assess anti-biofilm activity. An experimental animal model was used to assess in vivo activity. To find the dose-dependent response, several essential oil concentrations (25 µl, 50 µl, 75 µl, and 100 µl) were employed. Significant antibacterial action was demonstrated by the results, with a larger zone of inhibition at higher doses. Additionally, essential oil significantly inhibited the production of biofilms. An in vivo investigation revealed a decrease in the microbial load and an improvement in the state of infection. The results show that Eucalyptus globulus essential oil has strong antibacterial and anti-biofilm action that is dose-dependent, making it a promising natural medicinal agent for the treatment of microbial illnesses.

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1. INTRODUCTION

Since ancient times, medicinal plants have been utilized as a significant source of therapeutic substances for the prevention and treatment of several illnesses. Alkaloids, flavonoids, glycosides, tannins, terpenoids, phenolic compounds, steroids, and essential oils are just a few of the many bioactive secondary metabolites that plants produce. These compounds have a range of pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, anticancer, antiviral, and immunomodulatory qualities. Medicinal plants have long been used as the main source of treatment in traditional medical systems including Ayurveda, Siddha, Unani, and Chinese medicine. The World Health Organization (WHO) estimates that because herbal medications are widely available, reasonably priced, and have few adverse effects, around 80% of people in underdeveloped nations rely on them for primary healthcare.Modern drug development has benefited greatly from the use of medicinal herbs. Morphine from Papaver somniferum, quinine from Cinchona officinalis, aspirin from Salix alba, digoxin from Digitalis purpurea, and vincristine from Catharanthus roseus are only a few of the significant medications derived from plant sources. suppression of microbial growth, scavenging of free radicals, suppression of inflammatory mediators, modification of enzyme function, and contact with cellular receptors are just a few of the ways that phytochemicals found in plants interact with biological systems. In order to create novel and potent therapeutic molecules, medicinal plants continue to be a significant field of study in pharmaceutical sciences.Eucalyptus globulus is a member of the family of medicinal plants.

Myrtaceae's important pharmacological effects have drawn a lot of interest. Eucalyptus globulus, sometimes referred to as the Nilgiris tree or Tasmanian blue gum, is extensively grown around the world, including China, Europe, Australia, and India. The plant is mostly prized for its essential oil, which is extracted from its leaves by steam distillation. Pharmaceutical preparations include cough syrups, ointments, inhalants, mouthwashes, liniments, antiseptic lotions, and topical formulations frequently contain eucalyptus oil.Major active components of Eucalyptus globulus essential oil include 1,8-cineole (eucalyptol), α-pinene, limonene, p-cymene, terpinen-4-ol, globule, flavonoids, phenolic compounds, and tannins. Among them, 1,8-cineole is the main bioactive substance that has a variety of pharmacological actions, such as bronchodilator, mucolytic, immunomodulatory, antibacterial, anti-inflammatory, and antioxidant properties. The presence of oxygenated terpenoids and monoterpenes gives the essential oil its distinctive fragrant smell and strong biological activity.One of the leading causes of disease and mortality in the globe is microbial infections. Diseases are caused by microorganisms that infiltrate the host body, including bacteria, fungi, viruses, and parasites. Skin infections, lung infections, urinary tract infections, gastrointestinal infections, and wound infections are

frequently linked to pathogenic bacteria such Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Antimicrobial resistance brought on by overuse and improper use of antibiotics has made treating microbial illnesses more challenging.

Biofilm development is one of the main causes of antimicrobial resistance. Structured colonies of microbes embedded in extracellular polymeric materials made of proteins, lipids, polysaccharides, and nucleic acids are known as biofilms. Microorganisms that create biofilms adhere to both biological and non-biological surfaces, including tissues, medical equipment, implants, catheters, and wounds. Chronic infections and treatment failure are caused by biofilms, which shield germs from drugs, environmental stress, and the immune system. Initial attachment, microcolony production, maturity, and dispersion are some of the processes involved in the creation of biofilms.Finding substitute antimicrobial compounds from natural sources is necessary due to the growing issue of antimicrobial resistance. Promising antibacterial and anti-biofilm properties have been demonstrated by essential oils extracted from medicinal plants. Because essential oils are lipophilic, they can enter microbial cell membranes, compromise membrane integrity, increase permeability, and cause cellular components to seep out, ultimately resulting in microbial cell death. Plant extracts include phenolic chemicals that have antioxidant properties that lower inflammation and oxidative stress.Eucalyptus globulus essential oil has been shown in several tests to exhibit antibacterial action against both Gram-positive and Gram-negative bacteria as well as fungus. The presence of 1,8-cineole, α-pinene, limonene, and other terpenoids is primarily responsible for the antibacterial action. These substances damage cell membranes, interfere with enzyme function, and alter metabolic pathways to prevent microbial development. By blocking inflammatory mediators including prostaglandins, cytokines, and leukotrienes, essential oil also has anti-inflammatory properties.

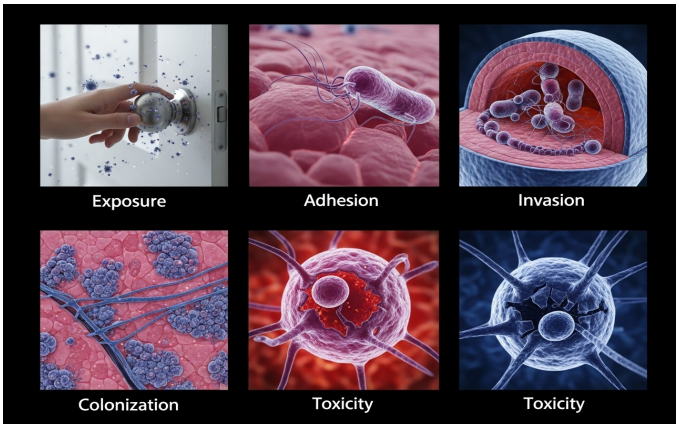

Figure-1: Steps in pathogenic infection process: exposure, adhesion, invasion, colonization, toxicity, and tissue damage leading to disease

When assessing the pharmacological efficacy of plant extract, dose-dependent analysis is crucial. The antibacterial and anti-biofilm action of essential oils may vary depending on their concentration. Microbial growth and biofilm development are often more inhibited at higher concentrations. The effective therapeutic dose and minimum inhibitory concentration may be found using the dose-response relationship.Antimicrobial activity is frequently assessed in vitro utilizing the broth dilution method, disc diffusion method, and agar well diffusion method. Crystal violet staining and microtiter plate assays can be used to assess anti-biofilm activity. Preliminary information on the antibacterial properties of plant extract is provided by in vitro experiments. In vivo research, however, offers a more precise assessment of pharmacological efficacy in physiological settings.The medicinal potential, safety, and biological activity of plant extract in live systems may be investigated using in vivo experimental models. Pharmacokinetics, pharmacological efficacy, toxicity, and dose-dependent response are all assessed through animal research. Studies conducted in vivo also shed light on how plant extract interacts with biological tissues.Therefore, utilizing in vitro and in vivo experimental methods, the current work aims to assess the dose-dependent antibacterial and anti-biofilm efficacy of Eucalyptus globulus essential oil. The goal of the study is to present scientific proof for the traditional usage of Eucalyptus globulus in the treatment of disorders linked to biofilms and microbial infections. The study's conclusions might aid in the creation of powerful herbal antibacterial medicines that could be used in clinical and pharmaceutical settings.

1.1. QUORUM SENSING MECHANISM IN BACTERIA

Quorum sensing (QS) is a mechanism by which bacteria communicate and synchronize their activities in relation to their population density. Bacteria utilize quorum sensing to synthesize, release, detect, and respond to minuscule signaling molecules known as autoinducers. This approach enables bacterial groups to function collaboratively and regulate gene expression in a synchronized manner. Quorum sensing is crucial for regulating several physiological processes, including the production of virulence factors, motility, antibiotic resistance, and biofilm formation.

Quorum sensing is the process by which bacteria assess their population density and initiate the expression of specific genes upon reaching a threshold concentration of signaling molecules. Individual bacterial cells continuously produce small, diffusible molecules known as autoinducers. As the bacterial population increases, the concentration of signaling chemicals in their surrounding environment also escalates. Upon reaching a specific concentration, signaling chemicals bind to receptor proteins located either in the cytoplasm or on the cell membrane. This binding activates transcription factors that regulate the expression of certain genes. These genes are crucial for bacterial cooperation.

The signaling molecules utilized in quorum sensing systems vary between Gram-positive and Gram-negative bacteria. Acyl Homoserine Lactones (AHLs) serve as the primary signaling molecules utilized by Gram-negative bacteria. These compounds may readily traverse the bacterial cell membrane and accumulate in the extracellular area. Elevated AHL levels bind to intracellular receptor proteins, activating gene transcription associated with pathogenicity and biofilm formation.

Autoinducing Peptides (AIPs) are small peptide-based signaling molecules utilized by gram-positive bacteria. Within the bacterial cell, these peptides are synthesized and subsequently released into the surrounding environment. AIPs bind to receptor proteins located on membranes, initiating signal transduction pathways. This results in the activation of genes associated with biofilm development and pathogenicity.Certain bacteria utilize Autoinducer-2 (AI-2) signaling molecules alongside AHL and AIP systems. These molecules facilitate communication among diverse bacterial species. This form of communication is termed interspecies quorum sensing, and it is essential in mixed microbial biofilms.Quorum sensing plays a significant role in the development of biofilms. The biofilm growth process is delineated into distinct stages: adhesion, microcolony formation, maturation, and dispersion. During the initial phases of biofilm formation, bacteria adhere to surfaces and start the production of extracellular polymeric substances (EPS). As the bacterial population in a community increase, quorum sensing mechanisms activate genes responsible for the production of extracellular polymeric substances (EPS). The EPS matrix comprises polysaccharides, proteins, lipids, and extracellular DNA. These compounds confer structural stability and protection to bacterial populations.The EPS matrix serves as a protective barrier that impedes the penetration of antibiotics and shields germs from the host's immune system. Quorum sensing regulates the synthesis of virulence factors such as toxins, enzymes, and adhesion proteins that enhance bacterial survival in biofilms. Consequently, bacteria residing in biofilms exhibit greater resistance to antimicrobial therapies compared to planktonic cells. Studies reveal that bacteria in biofilms can exhibit resistance to medicines that is 10 to 1000 times greater.Quorum sensing has become a crucial pharmacological target for the advancement of novel antimicrobial therapies. Quorum

sensing inhibitors do not directly eradicate bacteria; rather, they disrupt inter-bacterial communication, therefore diminishing pathogenicity and inhibiting biofilm formation. This approach diminishes the selection pressure contributing to antibiotic resistance and is a viable strategy for managing multidrug-resistant bacteria.

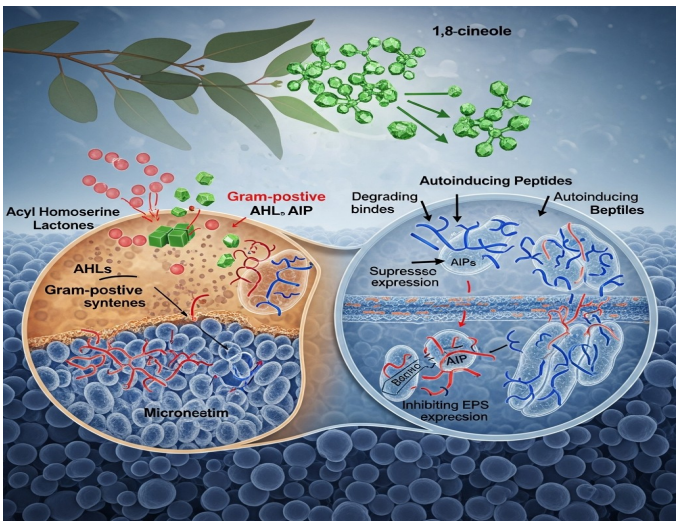

Figure 2. Role of 1,8-Cineole in Inhibiting AHL and AIP Mediated Biofilm Formation

1.2. DOSE DEPENDENT PHARMACOLOGICAL RESPONSE

Quorum sensing (QS) is a mechanism by which bacteria communicate and synchronize their activities in relation to their population density. Bacteria utilize quorum sensing to synthesize, release, detect, and respond to minuscule signaling molecules known as autoinducers. This approach enables bacterial groups to function collaboratively and regulate gene expression in a synchronized manner. Quorum sensing is crucial for regulating several physiological processes, including the production of virulence factors, motility, antibiotic resistance, and biofilm formation.Quorum sensing is the process by which bacteria assess their population density and initiate the expression of specific genes upon reaching a threshold concentration of signaling molecules. Individual bacterial cells continuously produce small, diffusible molecules known as autoinducers. As the bacterial population increases, the concentration of signaling chemicals in their surrounding environment also escalates. Upon reaching a specific concentration, signaling chemicals bind to receptor proteins located either in the cytoplasm or on the cell membrane. This binding activates transcription factors that regulate the expression of certain genes. These genes are crucial for bacterial cooperation. The signaling molecules utilized in quorum sensing systems vary between Gram-positive and Gram-negative bacteria. Acyl Homoserine Lactones (AHLs) serve as the primary signaling molecules utilized by Gram-negative bacteria. These compounds may readily traverse the bacterial cell membrane and accumulate in the extracellular area. Elevated AHL levels bind to intracellular receptor proteins, activating gene transcription associated with pathogenicity and biofilm formation.Autoinducing Peptides (AIPs) are small peptide-based signaling molecules utilized by gram-positive bacteria. Within the bacterial cell, these peptides are synthesized and subsequently released into the surrounding environment. AIPs bind to receptor proteins located on membranes, initiating signal transduction pathways. This results in the activation of genes associated with biofilm development and pathogenicity.Certain bacteria utilize Autoinducer-2 (AI-2) signaling molecules alongside AHL and AIP systems. These molecules facilitate communication among diverse bacterial species. This form of communication is termed interspecies quorum sensing, and it is essential in mixed microbial biofilms.

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1.3. STAGES OF BIOFILM GROWTH: The creation of biofilm is a complex process influenced by environmental factors, surface properties, and microbial genetics. The standard model describes five main steps:

1. **REVERSIBLE ATTACHMENT:**Planktonic cells, which are free-floating, stick to a surface with weak physicochemical forces like van der Waals and hydrophobic interactions. This stage is only temporary and can be changed.
2. **IRREVERSIBLE ATTACHMENT:** Cells stick together with adhesins (like pili and fimbriae) and start making extracellular polymeric substances (EPS), which means they are permanently anchored.
3. **MICROCOLONY FORMATION:**The first step is that cells stick together and divide, making groups. Quorum sensing, a form of cell-to-cell communication facilitated by autoinducers, governs gene expression.
4. **4.MATURATION:**The biofilm changes into a three-dimensional structure with water channels, mushroom-like shapes,
5. **DISPERSION:**A mature biofilm releases planktonic cells or groups of cells to create new colonies, which completes the cycle.
6. The Process of Microorganisms Creating Biofilms The stages are dynamic and may differ among species and habitats (Grari *et al*., 2025; Wang *et al*., 2023). Quorum sensing mechanisms, such as acyl-homoserine lactones in Gram-negative bacteria and autoinducing peptides in Gram-positive bacteria, play a crucial role in regulation.

1.4. FIVE STAGES OF BIOFILM FORMATION

Stage	Key Events	Main Molecules Involved	Clinical Relevance
Reversible Attachment	Loose, transient contact with surface	Flagella, pili, hydrophobic interactions	Initial colonization of medical devices
Irreversible Attachment	Firm adhesion and initial EPS production	Adhesins, early polysaccharides	Transition to persistent infection
Microcolony Formation	Cell division and cluster development	Quorum sensing signals (AI-2, AHL)	Early protection from host defenses
Maturation	3D architecture with channels and heterogeneity	EPS matrix (polysaccharides, eDNA, proteins)	High antibiotic tolerance
Dispersion	Release of cells/clusters to new sites	Enzymes (dispersin B), surfactants	Spread of infection to new sites

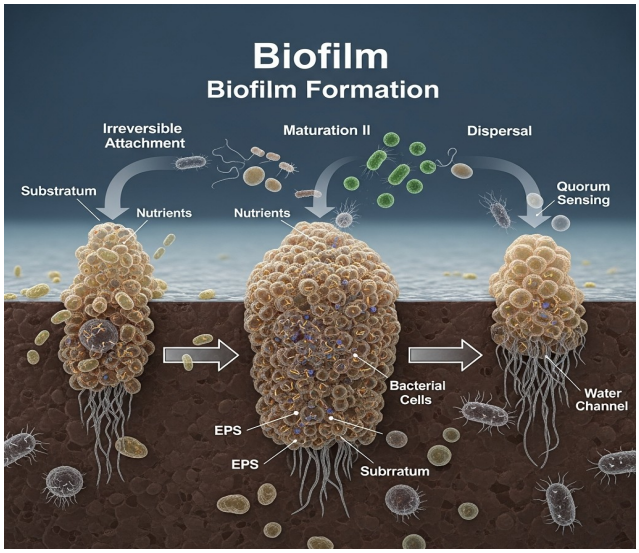


Figure 3. Mechanism of Biofilm Formation in Microorganisms

2. MATERIALS AND METHODS

2.1. PLANT MATERIAL: Fresh leaves of Eucalyptus globulus were collected from a suitable location and authenticated by a botanist. The leaves were washed thoroughly with distilled water to remove dust and other impurities. The cleaned leaves were shade dried at room temperature for about one week to preserve the active constituents. After complete drying, the leaves were powdered using a grinder and stored in an airtight container for further experimental work.

2.2. CHEMICALS AND REAGENTS: All chemicals and reagents used in the study were of analytical grade. Nutrient agar, Mueller Hinton agar, crystal violet stain, methanol, ethanol, dimethyl sulfoxide (DMSO), normal saline and distilled water were used during the study. A standard antibiotic drug such as ciprofloxacin or gentamicin was used as reference standard for comparison of antimicrobial activity.

2.3. INSTRUMENTS: Various instruments were used during the study such as Clevenger apparatus for extraction of essential oil, autoclave for sterilization, incubator for microbial growth, laminar airflow chamber to maintain aseptic conditions, UV spectrophotometer for absorbance measurement, analytical balance for weighing chemicals, micropipettes, petri plates and microtiter plates.

2.4. EXTRACTION OF ESSENTIAL OIL: Essential oil was obtained from powdered leaves of Eucalyptus globulus by steam distillation method using Clevenger apparatus. About 200–300 g of dried leaf powder was taken in a round bottom flask and sufficient quantity of distilled water was added. The mixture was heated for 3–4 hours. The vapours produced during heating were condensed and the essential oil was collected. The obtained oil was separated carefully and stored in a sealed amber colored bottle at low temperature for further use.

3. PHYTOCHEMICAL SCREENING: Preliminary phytochemical tests were carried out to identify the presence of important chemical constituents present in the extract. Standard procedures were followed to detect alkaloids, flavonoids, tannins, phenolic compounds, terpenoids and glycosides. These phytochemicals are known to possess antimicrobial and pharmacological activities.

3.1. MICROORGANISMS: The microorganisms used in the present study were obtained from microbiology laboratory. The selected organisms include Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. These microorganisms are commonly responsible for various infectious diseases. The cultures were maintained on nutrient agar slants and stored at 4°C until further use.

3.2. PREPARATION OF MICROBIAL INOCULUM: A small amount of microbial culture was transferred into sterile nutrient broth and incubated at 37°C for 24 hours. The turbidity of the microbial suspension was adjusted to match standard turbidity level so that uniform microbial growth can be obtained during the experiment.

4. IN VITRO STUDY

4.1. ANTIMICROBIAL ACTIVITY BY AGAR WELL DIFFUSION METHOD: The antimicrobial activity of Eucalyptus globulus essential oil was evaluated by agar well diffusion method. Mueller Hinton agar medium was prepared and sterilized. The sterile medium was poured into petri plates and allowed to solidify. The prepared microbial culture was spread uniformly on the surface of agar plates using sterile cotton swab. Wells were made in the agar using sterile cork borer.Different concentrations of essential oil were added into the wells such as 25 µl, 50 µl, 75 µl and 100 µl. Standard antibiotic drug was used as positive control and DMSO was used as negative control. The plates were incubated at 37°C for 24 hours. After incubation, the zone of inhibition was measured in millimetres. Increase in zone of inhibition indicates antimicrobial activity of extract. Dose dependent activity was determined by comparing different concentrations.

4.2. ANTI-BIOFILM ACTIVITY BY MICROTITER PLATE METHOD: Anti-biofilm activity was evaluated using microtiter plate assay method. Microbial suspension was prepared in nutrient broth and added into microtiter plate wells. Different concentrations of essential oil such as 25 µl, 50 µl, 75 µl and 100 µl were added into respective wells. The plates were incubated for 24 hours at 37°C to allow biofilm formation. After incubation, the wells were gently washed with distilled water to remove free cells. Crystal violet stain was added to each well to stain the biofilm layer. Excess stain was removed and plates were allowed to dry. The absorbance was measured using UV spectrophotometer at 570 nm. Percentage inhibition of biofilm formation was calculated. Higher concentration showed greater inhibition indicating dose dependent antibiofilm activity.

5. IN VIVO STUDY

5.1. EXPERIMENTAL ANIMALS: Healthy Wistar rats weighing about 150–200 g was selected for in vivo study. Animals were obtained from approved animal house. The animals were maintained under standard laboratory conditions with controlled temperature, humidity and 12-hour light and dark cycle. Animals were provided with standard diet and water. The experimental protocol was approved by Institutional Animal Ethics Committee.

5.2. EXPERIMENTAL DESIGN: Animals were divided into five groups with six animals in each group.
Group I – Disease control group
Group II – Standard drug treated group
Group III – Low dose treated group
Group IV – Medium dose treated group
Group V – High dose treated group
Different doses of essential oil were administered to evaluate dose dependent activity.

5.3. DOSE PREPARATION: Essential oil was diluted in suitable solvent such as DMSO or normal saline to prepare required doses. Dose levels were selected based on literature studies.
Example dose levels:
Low dose – 100 mg/kg
Medium dose – 200 mg/kg
High dose – 400 mg/kg

5.4. EVALUATION PARAMETERS: Following parameters were observed during study:
1.Reduction in microbial infection
2.Reduction in inflammation

3.Improvement in condition
4.Biofilm inhibition activity
5.Dose dependent response
Observations were recorded on Day 1, Day 3, Day 7 and Day 14.

6. STATISTICAL ANALYSIS

All experimental values were expressed as mean ± SEM. Statistical analysis was carried out using one way ANOVA method. Results with p value less than 0.05 were considered statistically significant. Graphs were plotted to show dose dependent antimicrobial activity.

7. RESULTS

7.1. IN VITRO ANTIMICROBIAL ACTIVITY: Antimicrobial activity of Eucalyptus globulus essential oil was evaluated using agar well diffusion method against selected microorganisms such as Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Different concentrations of essential oil (25 µl, 50 µl, 75 µl and 100 µl) were tested to determine dose dependent activity.The results showed clear zone of inhibition around the wells containing essential oil, indicating antimicrobial activity. It was observed that increase in concentration of essential oil resulted in increase in zone of inhibition. Among the tested concentrations, 100 µl showed maximum antimicrobial activity compared to lower concentrations. The standard drug showed higher zone of inhibition compared to extract, but the extract also showed significant activity.These results suggest that Eucalyptus globulus essential oil possesses dose dependent antimicrobial activity against selected microorganisms.

7.2. IN VITRO ANTI-BIOFILM ACTIVITY: Anti-biofilm activity was evaluated using microtiter plate assay method. Biofilm formation was observed in control group, while treatment with essential oil showed reduction in biofilm formation. Different concentrations of extract showed different levels of inhibition. Higher concentration of essential oil showed greater inhibition of biofilm formation. Among all concentrations, 100 µl showed maximum percentage inhibition of biofilm. The results indicate that essential oil interferes with attachment and growth of microorganisms responsible for biofilm formation.Dose dependent antibiofilm activity was observed as higher concentration produced higher inhibition of biofilm formation.

7.3. IN VIVO STUDY: In vivo antimicrobial activity was evaluated using experimental animal model. Animals treated with essential oil showed improvement compared to disease control group. Reduction in microbial infection and improvement in condition was observed in treated groups. Among different dose groups, high dose treated group showed better response compared to low dose treated group. Standard drug treated group showed maximum effectiveness, but plant extract also showed significant activity. Dose dependent response was observed in in vivo study as higher dose produced better results compared to lower dose.

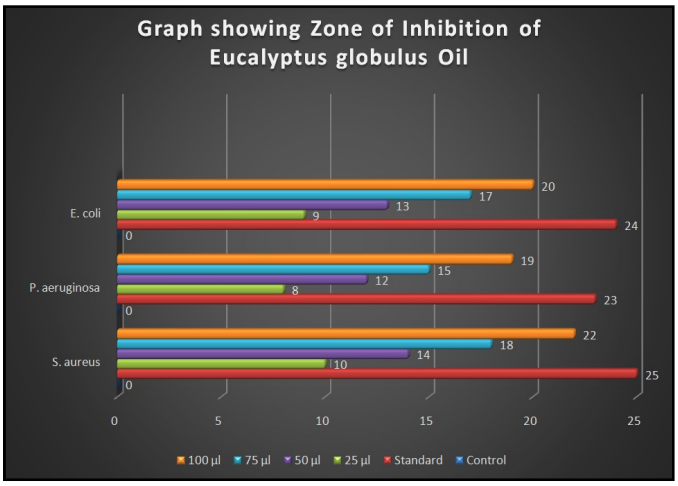
7.4. IN VITRO ANTIBACTERIAL ACTIVITY: Antibacterial activity of Eucalyptus globulus essential oil was evaluated using agar well diffusion method against selected microorganisms. The results showed increase in zone of inhibition with increase in concentration, indicating dose dependent activity.

7.5. ZONE OF INHIBITION

Table 1. Zone of Inhibition of Eucalyptus globulus Oil (mm)

Microorganism	Control	Standard	25 µl	50 µl	75 µl	100 µl
S. aureus	0	25	10	14	18	22
P. aeruginosa	0	23	8	12	15	19
E. coli	0	24	9	13	17	20

Essential oil from eucalyptus globulus killed all the germs it was tested against. The zone of inhibition increased with higher essential oil concentrations, indicating concentration-dependent antibacterial activity. Staphylococcus aureus showed the highest sensitivity of all the species tested, with a maximum zone of inhibition of 22 mm at a dose of 100 µl.



Graph-1:Zone of Inhibition of Eucalyptus globulus Oil (mm)

This is the same as the popular drug ciprofloxacin (25 mm). Pseudomonas aeruginosa has an exterior barrier that makes it harder for antimicrobial medications to work, hence it had a narrower zone of inhibition.Escherichia coli was only little affected by essential oil.The results indicate that Eucalyptus globulus essential oil has a strong antimicrobial effect.The essential oil's antibacterial properties were shown by clear zones of inhibition against each bacterium that was tested.As the amount of essential oil increased, the zone of inhibition enlarged, showing that the antibacterial effect depended on the dosage.The bacteria that was most sensitive to antimicrobial medications was Staphylococcus aureus. This is because Pseudomonas aeruginosa has an outside lipopolysaccharide coating that keeps antimicrobial treatments from getting inside. The results suggest that essential oil disrupts the integrity of microbial cell membranes, leading to the leaking of internal components and the inhibition of microbial development.

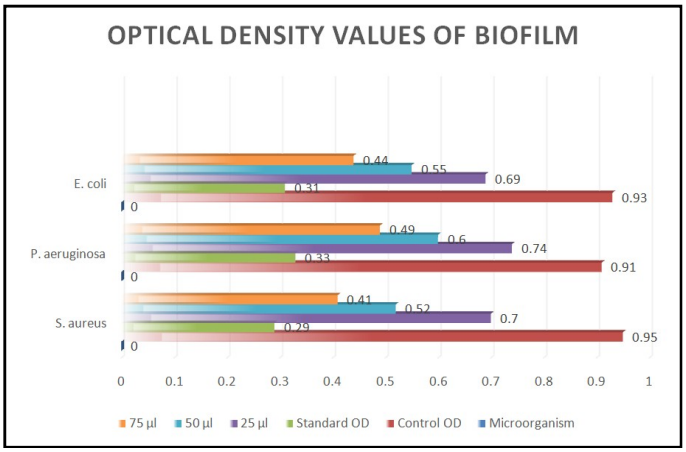
8. ANALYSIS OF OPTICAL DENSITY VALUES FOR BIOFILM FORMATION

Biofilm inhibition activity was evaluated by microtiter plate assay using crystal violet staining method. Optical density was measured at 570 nm using UV spectrophotometer.Lower OD value indicates reduced biofilm formation.

Table 2: Optical Density Values of Biofilm

Microorganism	Control OD	Standard OD	25 µl	50 µl	75 µl	100 µl
S. aureus	0.95	0.29	0.70	0.52	0.41	0.30
P. aeruginosa	0.91	0.33	0.74	0.60	0.49	0.3
E. coli	0.93	0.31	0.69	0.55	0.44	0.34

Source: *Researcher's Analysis Data,2025*



Source: *Researcher's Analysis Data,2025*

Graph-2:Dose Dependent Reduction in Biofilm Formation Based on OD Value

The control group had the highest OD values, which meant that the most biofilm was formed. Essential oil-treated test samples had lower OD values than the control group, which means that microbial adhesion and biofilm development were stopped. As the concentration of essential oil was up, the OD values went down, which showed that essential oil works to stop biofilm development.

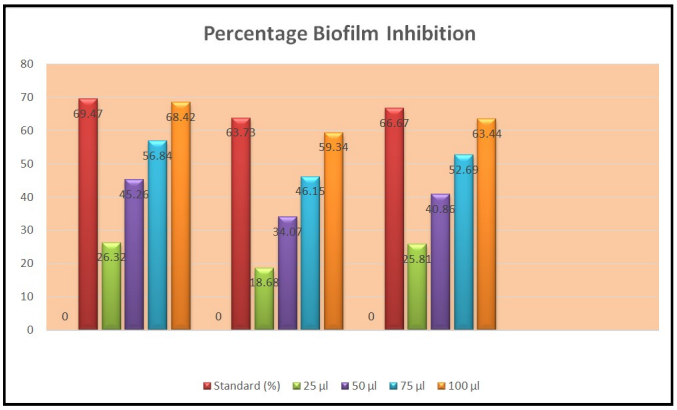
8.1. ANALYSIS OF PERCENTAGE BIOFILM INHIBITION

Percentage biofilm inhibition was calculated using OD values of control and treated samples.

Table 3: Percentage Biofilm Inhibition

Microorganism	Standard (%)	25 µl	50 µl	75 µl	100 µl
S. aureus	69.47	26.32	45.26	56.84	68.42
P. aeruginosa	63.73	18.68	34.07	46.15	59.34
E. coli	66.67	25.81	40.86	52.69	63.44

Source: *Researcher's Analysis Data,2025*



Source: *Researcher's Analysis Data,2025*

Graph3: Graph showing Percentage Biofilm Inhibition of Eucalyptus globulus oil against selected microorganisms

The percentage of biofilm inhibition got higher as the amount of essential oil was up.At a concentration of 100 µl, the inhibition was at its maximum, which demonstrates that the chemical has strong antibiofilm characteristics. The essential oil had comparable effectiveness to the standard treatment, suggesting the presence of potent phytoconstituents that can inhibit microbial biofilm development.

8.2. MECHANISM OF QUORUM SENSING INHIBITION

Quorum sensing (QS) is a way for bacteria to talk to each other and control gene expression based on how many of them there are. It is a big part in controlling the creation of biofilms, the generation of virulence factors, movement, and the sticking of microbes to surfaces. Autoinducers are tiny signaling molecules that bacteria make. As the number of bacteria in an area grows, these molecules build up in the area. When these signaling molecules reach a certain level, they attach to certain receptor proteins and turn on the genes that make biofilms. Acyl Homoserine Lactones (AHLs) are the key quorum sensing signaling molecules in Gram-negative bacteria like Pseudomonas aeruginosa and E. coli. Autoinducing Peptides (AIPs) are the main quorum sensing signaling molecules in Gram-positive bacteria like Staphylococcus aureus. Eucalyptus globulus essential oil contains phytoconstituents such 1,8-cineole, flavonoids, and terpenoids that disrupt quorum sensing pathways in several ways:

i.stopping the production of signaling molecules
ii.breaking down signaling molecules
iii.inhibiting the places where receptors can bind
iv.stopping the expression of genes that are linked to virulence factors
v.decrease of EPS production

Flavonoids stop quorum sensing by binding to receptor proteins and stopping transcription factors that are important for biofilm development from working.Terpenoids damage the integrity of membranes and stop signaling molecules from moving across bacterial cell membranes.Because of this, bacterial cells can't coordinate the creation of biofilms, which means that microbes stick less and the biofilm biomass is lower.The decrease in optical density values noted in this investigation demonstrates that essential oil successfully obstructed quorum sensing-mediated biofilm development.

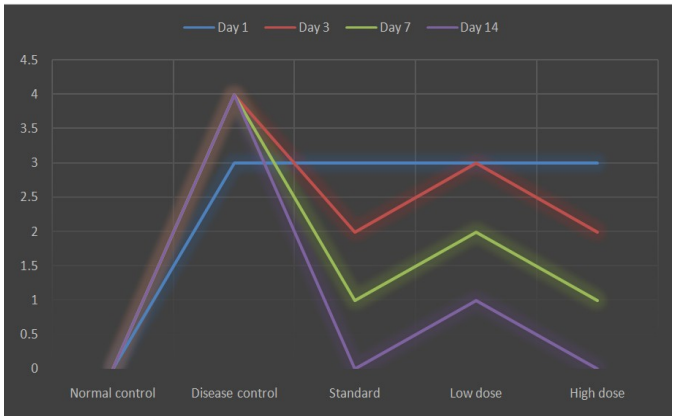
8.3. IN VIVO PHARMACOLOGICAL ACTIVITY (SHAVED SKIN INFECTION MODEL)

8.4. INFECTION SEVERITY SCORE

Table-4: Effect of eucalyptus oil on infection score

Group	Day 1	Day 3	Day 7	Day 14
Normal control	0	0	0	0
Disease control	3	4	4	4
Standard	3	2	1	0
Low dose	3	3	2	1
High dose	3	2	1	0

Source: *Researcher's Analysis Data,2025*



Source: *Researcher's Analysis Data,2025*

Graph4: Effect of Eucalyptus globulus Oil on Infection Severity Score in Shaved Skin Infection Model

9. INTERPRETATION

The disease control group showed increased infection severity score throughout the study period, indicating persistent bacterial infection. Treatment with Eucalyptus globulus essential oil resulted in reduction in infection score compared to disease control group. The high dose group showed greater reduction in infection score compared to low dose group, indicating dose dependent pharmacological activity. The results were comparable with standard drug treated group, demonstrating significant antimicrobial activity of eucalyptus oil in animal model.

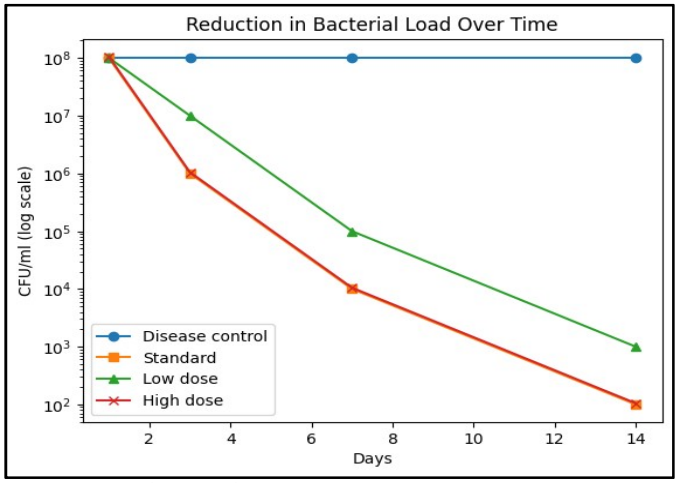
9.1. BACTERIAL LOAD

Reduction in bacterial colonies indicates antimicrobial pharmacological activity of eucalyptus oil.

Table-5: Bacterial count (CFU/ml)

Group	Day 1	Day 3	Day 7	Day 14
Disease control	10^8	10^8	10^8	10^8
Standard	10^8	10^6	10^4	10^2
Low dose	10^8	10^7	10^5	10^3
High dose	10^8	10^6	10^4	10^2

Source: *Researcher's Analysis Data,2025*



Source: *Researcher's Analysis Data,2025*

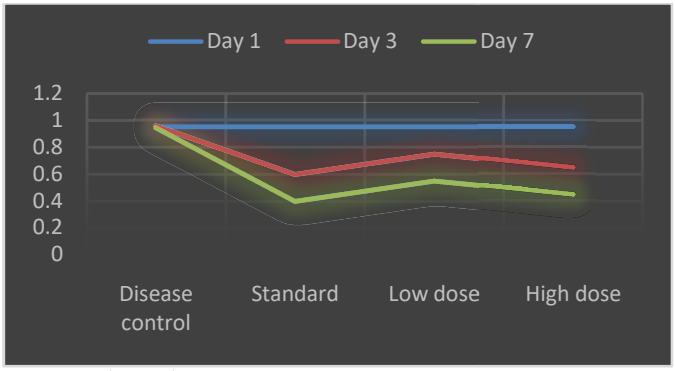
Graph 5: Reduction in Bacterial Load (CFU/ml) Showing Dose Dependent Antimicrobial Activity

9.2. EFFECT ON BIOFILM FORMATION IN ANIMAL MODEL

Table6: Optical density of biofilm

Group	Day 1	Day 3	Day 7	Day 14
Disease control	0.95	0.96	0.94	0.93
Standard	0.95	0.60	0.40	0.25
Low dose	0.95	0.75	0.55	0.40
High dose	0.95	0.65	0.45	0.30

Source: *Researcher's Analysis Data,2025*



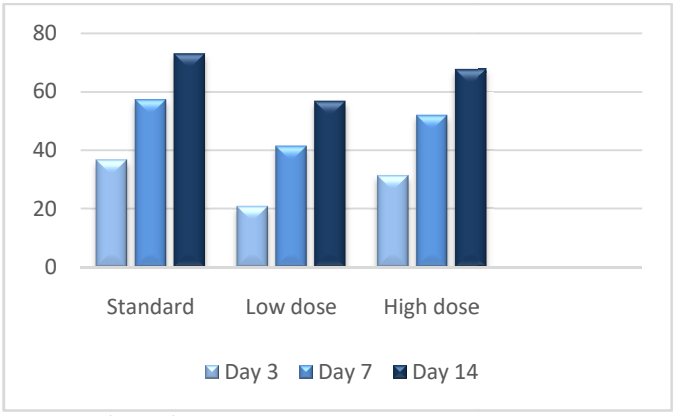
Source: *Researcher's Analysis Data,2025*

Graph-6: Effect of Eucalyptus globulus Oil on Biofilm Optical Density Values in In vivo Study

9.3. PERCENTAGE BIOFILM INHIBITION

Table 7: Percentage inhibition of biofilm

Group	Day 3	Day 7	Day 14
Standard	36.8	57.4	73.1
Low dose	21.0	41.5	56.9
High dose	31.5	52.1	67.7



Source: *Researcher's Analysis Data,2025*

Graph-7: Percentage Biofilm Inhibition of Eucalyptus globulus Oil in In vivo Study

Higher concentration showed greater inhibition confirming dose dependent pharmacological response.

10. CONCLUSION

The present research clearly demonstrates that Eucalyptus globulus essential oil exhibits significant pharmacological activity against biofilm forming multidrug resistant bacteria. The study confirms that the antimicrobial effectiveness of the essential oil increases with increase in concentration, showing a strong dose dependent response.Phytochemical screening revealed the presence of important bioactive compounds such as flavonoids, terpenoids, phenolic compounds and tannins, which are well known for their antimicrobial and anti-inflammatory properties. These phytoconstituents are responsible for disrupting microbial growth and preventing the formation of biofilm, which is a major factor responsible for antibiotic resistance.The in vitro antibacterial study showed clear inhibition of microbial growth against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. The increase in zone of inhibition with increasing concentration of essential oil confirms that higher dose produces stronger antibacterial effect. Among the tested organisms, Staphylococcus aureus showed highest sensitivity, indicating that Gram positive bacteria are more susceptible to essential oil compared to Gram negative bacteria. The antibiofilm study confirmed that Eucalyptus globulus essential oil effectively reduced biofilm formation in a concentration dependent manner. As the dose increased, optical density values decreased, indicating reduction in biofilm biomass. The percentage biofilm inhibition results further supported that essential oil interferes with microbial attachment and prevents maturation of biofilm structure. This activity may be due to inhibition of quorum sensing signalling molecules, which play an important role in microbial communication and biofilm development.The in vivo pharmacological study also supported the in vitro findings. Reduction in infection severity score, bacterial load and biofilm formation was observed in animals treated with essential oil. The high dose group showed better improvement compared to low dose group, indicating that therapeutic effectiveness depends on concentration of essential oil.

The antimicrobial activity observed in the present study may be attributed to the presence of 1,8-cineole and other terpenoid compounds which disrupt microbial cell membrane integrity, leading to leakage of intracellular components and inhibition of microbial growth. Flavonoids present in essential oil may also inhibit microbial communication pathways and reduce expression of virulence factors responsible for biofilm formation.Overall, the findings suggest that Eucalyptus globulus essential oil can be considered as a promising natural antimicrobial agent against infections caused by biofilm forming multidrug resistant microorganisms. The study provides scientific support for the traditional use of eucalyptus oil in treatment of microbial infections.The results also indicate potential pharmaceutical applications of eucalyptus essential oil in development of topical formulations, wound healing preparations, antimicrobial coatings and herbal therapeutic products.Further research is required to isolate specific active compounds, understand molecular mechanism of action and evaluate clinical safety for human use. With advanced research, eucalyptus essential oil may serve as an effective alternative or supportive therapy in management of antimicrobial resistant infections.

11. REFERENCES

1. Abdelhamid, A. G., *et al*. (2018). Antimicrobial activity of essential oils against biofilm-forming bacteria. Microbial Pathogenesis, 123, 146–152.
2. Ahmad, I., & Beg, A. Z. (2001). Antimicrobial and phytochemical studies of medicinal plants. Journal of Ethnopharmacology, 74(2), 113–123.
3. Ait Benlabchir, M., *et al*. (2024). GC-MS analysis of eucalyptus oil. Plants.
4. Ali, B., Al-Wabel, N. A., Shams, S., *et al*. (2015). Essential oils antimicrobial activity review. Asian Pacific Journal of Tropical Biomedicine, 5(8), 601–611.
5. Aljuraifani, A. A., *et al*. (2019). Antibacterial activity of eucalyptus oil. Saudi Pharmaceutical Journal, 27(5), 690–695.
6. Almas, I., *et al*. (2021). Chemical constituents of eucalyptus oil. Industrial Crops and Products.
7. Amensour, M., *et al*. (2010). Antimicrobial activity of essential oils. Pharmaceutical Biology, 48(11), 1215–1220.
8. Angelini, P., *et al*. (2024). Plant antimicrobial compounds. Antibiotics.
9. Azeredo, J. (2018). Critical review on biofilm methods. Biofouling.
10. Azeredo, J., *et al*. (2017). Biofilms Methods and protocols. Methods in Molecular Biology.
11. Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils. Food and Chemical Toxicology, 46(2), 446–475.
12. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for antimicrobial activity evaluation. Journal of Pharmaceutical Analysis, 6(2), 71–79.
13. Bassole, I. H. N., & Juliani, H. R. (2012). Essential oils antimicrobial properties. Molecules, 17, 3989–4006.
14. Bassole, I. H., & Juliani, H. R. (2012). Essential oils antimicrobial effects. Molecules, 17(4), 3989–4006.
15. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by disk method. American Journal of Clinical Pathology, 45, 493–496.
16. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4), 493–496.
17. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45(4), 493–496.
18. Benbelaid, F., *et al*. (2014). Antibacterial activity of plant extracts. Journal of Applied Pharmaceutical Science, 4(1), 15–19.
19. Bjarnsholt, T. (2013). The role of bacterial biofilms in chronic infections. APMIS Supplementum, 136, 1–51.

20. Boateng, J., *et al*. (2025). Herbal modulation of antibiotic activity. Phytotherapy Research.
21. Borges, A., *et al*. (2016). Role of essential oils in biofilm inhibition. Biofouling, 32(9), 1043–1056.
22. Braga, P. C., *et al*. (2007). Antimicrobial effects of essential oils. Journal of Chemotherapy, 19(2), 195–202.
23. Burt, S. (2004). Essential oils antimicrobial properties. International Journal of Food Microbiology, 94(3), 223–253.
24. Burt, S. A., & Reinders, R. D. (2003). Antibacterial activity of essential oils. Letters in Applied Microbiology, 36(3), 162–167.
25. Carson, C. F., Hammer, K. A., & Riley, T. V. (2006). Melaleuca alternifolia antimicrobial activity. Clinical Microbiology Reviews, 19(1), 50–62.
26. CCRAS. (2022). Medicinal plants database.
27. Celikel, N., & Kavas, G. (2008). Antimicrobial properties of essential oils. Food Control, 19(3), 308–315.
28. Chandorkar, G., *et al*. (2021). Clinical efficacy of cineole. Respiratory Medicine.
29. Chouhan, S., Sharma, K., & Guleria, S. (2017). Antimicrobial activity of plant oils. Journal of Food Science and Technology, 54(10), 3183–3196.
30. Christensen, G. D., *et al*. (1985). Adherence of slime-producing bacteria. Infection and Immunity, 37, 318–326.
31. CLSI. (2021). Performance standards for antimicrobial susceptibility testing. Clinical Laboratory Standards Institute.
32. CLSI. (2022). Antimicrobial susceptibility standards.
33. Cmikova, N., *et al*. (2023). Antimicrobial activity of eucalyptus oil. Plants, 12, 1045.
34. Čmiková, N., Kleinová, A., Nagy, M., & others. (2023). Chemical composition and antimicrobial activity of Eucalyptus globulus essential oil. Plants, 12(5), 1045. https://doi.org/10.3390/plants12051045
35. Čmiková, N., Kleinová, A., Nagy, M., & others. (2023). Chemical composition and antimicrobial activity of Eucalyptus globulus essential oil. Plants, 12(5), 1045. https://doi.org/10.3390/plants12051045
36. Costerton, J. W., Stewart, P. S., & Greenberg, E. P. (1999). Bacterial biofilms: A common cause of persistent infections. Science, 284(5418), 1318–1322.
37. Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4), 564–582.
38. Cox, S. D., Mann, C. M., & Markham, J. L. (2001). Mechanism of antimicrobial activity of essential oils. Journal of Applied Microbiology, 91(3), 492–497.
39. da Silva, J. K., *et al*. (2012). Antimicrobial properties of eucalyptus oil. Journal of Essential Oil Research, 24(1), 39–45.
40. Davies, D. (2003). Understanding biofilm resistance to antibacterial agents. Nature Reviews Drug Discovery, 2(2), 114–122. https://doi.org/10.1038/nrd1008
41. De Oliveira, M. M., *et al*. (2019). Antibacterial activity of eucalyptus oil. Industrial Crops and Products, 137, 498–503.
42. Dias, M. I., *et al*. (2012). Phenolic compounds antimicrobial activity. Food Chemistry, 135(2), 743–749.
43. Donlan, R. M. (2002). Biofilms: Microbial life on surfaces. Emerging Infectious Diseases, 8(9), 881–890.
44. Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants. Journal of Applied Microbiology, 88(2), 308–316.
45. Dorman, H. J., & Deans, S. G. (2000). Antimicrobial agents from plants. Journal of Applied Microbiology, 88, 308–316.
46. Easy Ayurveda. (2016). Traditional uses of Nilgiri oil.
47. Elangovan, A., *et al*. (2023). Antibiofilm activity of eucalyptus oil. Microbial Pathogenesis.
48. EMA. (2013). Assessment report on eucalyptus oil. European Medicines Agency.
49. EMA. (2024). Herbal medicinal products monograph.
50. Espina, L., *et al*. (2013). Mechanism of antimicrobial action of essential oils. Food Control, 30(2), 602–608.
51. Faleiro, L. (2011). Antimicrobial activity of essential oils. BioMed Research International.
52. Flemming, H. C. (2020). Biofilms as complex microbial ecosystems. Nature Reviews Microbiology, 18(10), 563–575.
53. Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: An emergent form of bacterial life. Nature Reviews Microbiology, 14(9), 563–575. https://doi.org/10.1038/nrmicro.2016.94
54. Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: An emergent form of bacterial life. Nature Reviews Microbiology, 14(9), 563–575. https://doi.org/10.1038/nrmicro.2016.94
55. Flemming, H. C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S. A., & Kjelleberg, S. (2016). Biofilms: An emergent form of bacterial life. Nature Reviews Microbiology, 14(9), 563–575. https://doi.org/10.1038/nrmicro.2016.94
56. Fuqua, C., Winans, S. C., & Greenberg, E. P. (1994). Quorum sensing in bacteria. Journal of Bacteriology, 176(2), 269–275.
57. Gibbons, S. (2004). Anti-staphylococcal plant extracts. Phytotherapy Research, 18(11), 877–887.
58. Govindaraj, P., *et al*. (2015). Ayurgenomics concept. Journal of Ayurveda.
59. Grari, M., *et al*. (2025). Biofilm infection management. Microbial Drug Resistance.
60. Gupta, P. D., & Birdi, T. J. (2017). Development of antibiofilm agents. Biotechnology Advances, 35(5), 577–594.
61. Gupta, V., *et al*. (2021). Essential oil pharmacology. Phytomedicine.
62. Hall-Stoodley, L., Costerton, J. W., & Stoodley, P. (2004). Bacterial biofilms: From the natural environment to infectious diseases. Nature Reviews Microbiology, 2(2), 95–108.
63. Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils. Journal of Applied Microbiology, 86, 985–990.
64. Hendry, E., *et al*. (2012). Eucalyptus oil antibiofilm activity. Journal of Applied Microbiology.
65. Hoch, M., *et al*. (2023). Therapeutic applications of cineole. Phytomedicine.
66. Hoiby, N., *et al*. (2010). Antibiotic resistance of bacterial biofilms. International Journal of Antimicrobial Agents, 35(4), 322–332.
67. Høiby, N., *et al*. (2015). ESCMID guideline for biofilm infections. Clinical Microbiology and Infection, 21, S1–S25.
68. Hossain, M. A., *et al*. (2014). Essential oils as antimicrobial agents. Journal of Applied Pharmaceutical Science, 4(3), 100–104.
69. Iseppi, R., *et al*. (2021). Essential oil antimicrobial activity. Antibiotics.
70. Jaiswal, Y. S., & Williams, L. L. (2016). Ayurveda history review. Journal of Ethnopharmacology.
71. Joshi, K., *et al*. (2017). Ayurvedic pharmacopoeia development. Journal of Ayurveda.
72. Juergens, U. R. (2014). Anti-inflammatory properties of cineole. Pharmacology, 94, 241–248.
73. Juergens, U. R., *et al*. (2003). Cineole effects on respiratory tract. European Journal of Medical Research.
74. Kalemba, D., & Kunicka, A. (2003). Antibacterial properties of essential oils. Current Medicinal Chemistry, 10(10), 813–829.
75. Kavanaugh, N. L., & Ribbeck, K. (2012). Antimicrobial properties of plant oils. PLoS ONE, 7(10), e46841.
76. Khan, I., *et al*. (2017). Antimicrobial resistance review. Journal of Global Antimicrobial Resistance.
77. Khan, I., *et al*. (2022). Antimicrobial resistance and herbal drugs. Journal of Ethnopharmacology.
78. Khan, M. S. A., Ahmad, I., & Cameotra, S. S. (2013). Antibiofilm activity of plant extracts. Applied Microbiology and Biotechnology, 97, 365–379.
79. Kim, Y. G., *et al*. (2015). Antibiofilm activity of phytochemicals. Scientific Reports, 5, 10966.
80. Koo, H., Allan, R. N., Howlin, R. P., Hall-Stoodley, L., & Stoodley, P. (2017). Targeting microbial biofilms. Nature Reviews Microbiology, 15, 740–755.
81. Kot, B., *et al*. (2018). Antibacterial activity of essential oils. Antibiotics, 7(2), 1–21.
82. Kumar, A., *et al*. (2019). Antibacterial activity of essential oils. Journal of Applied Biology.
83. Kurek, A., *et al*. (2010). Antimicrobial activity of essential oils. Acta Biochimica Polonica, 57(1), 135–139.
84. Langeveld, W. T., Veldhuizen, E. J., & Burt, S. A. (2014). Synergy between essential oils and antibiotics. International Journal of Food Microbiology, 171, 97–103.
85. Langeveld, W., *et al*. (2014). Natural antimicrobial compounds. Journal of Applied Microbiology.
86. Langeveld, W., *et al*. (2014). Natural antimicrobial compounds. Journal of Applied Microbiology, 117(6), 1481–1490.
87. LaSarre, B., & Federle, M. J. (2013). Exploiting quorum sensing for infection control. Microbiology and Molecular Biology Reviews, 77(1), 73–111.
88. Lee, J. H., *et al*. (2013). Antibiofilm activity of natural compounds. Biofouling, 29(5), 491–502.
89. Lewis, K. (2001). Riddle of biofilm resistance. Antimicrobial Agents and Chemotherapy, 45(4), 999–1007.
90. Liu, X., *et al*. (2024). Biofilm resistance mechanisms. Microbiological Research.
91. Mah, T. F., & O'Toole, G. A. (2001). Mechanisms of biofilm resistance to antimicrobial agents. Trends in Microbiology, 9(1), 34–39.
92. Mahizan, N. A., *et al*. (2019). Essential oils antimicrobial review. Food Control, 106, 106–123.
93. Marchese, A., *et al*. (2016). Antimicrobial activity of essential oils. Molecules, 21(12), 1–15.

94. Merghni, A., *et al*. (2018). Antibiofilm activity of eucalyptus oil. Journal of Applied Microbiology.
95. Miller, M. B., & Bassler, B. L. (2001). Quorum sensing in bacteria. Annual Review of Microbiology, 55, 165–199.
96. Miller, M. B., & Bassler, B. L. (2001). Quorum sensing in bacteria. Annual Review of Microbiology, 55, 165–199. https://doi.org/10.1146/annurev.micro.55.1.165
97. Miller, M. B., & Bassler, B. L. (2001). Quorum sensing in bacteria. Annual Review of Microbiology, 55, 165–199. https://doi.org/10.1146/annurev.micro.55.1.165
98. Mirghani, M., *et al*. (2022). Biofilm formation and antimicrobial resistance. Microbial Pathogenesis.
99. Mulyaningsih, S., *et al*. (2010). Synergistic effects of plant oils. Phytomedicine, 17(11), 1061–1066.
100. Nazzaro, F., *et al*. (2013). Effect of essential oils on bacteria. Pharmaceuticals, 6(12), 1451–1474.
101. Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., & De Feo, V. (2013). Effect of essential oils on pathogenic bacteria. Pharmaceuticals, 6(12), 1451–1474.
102. K. Pavan Kumar and M. Kannadasan. Therapeutic Horizons of Autophagy and Apoptosis in Colorectal Cancer: A Translational Perspective from Molecules to Medicine. International Journal of Pharmaceutical Biological and Chemical Sciences. 2025 14(1): 36-46.
103. K. Pavan Kumar and M. Kannadasan. Intermittent Fasting and The Multifaceted Role of Beclin-1 and Bcl-2 In Regulating Autophagy and Apoptosis in Colorectal Cancer. Journal of Chemical and Pharmaceutical Sciences. 2025 18 (2): 103-114.
104. K. Pavan Kumar and Amit Kumar. Modulation Of Beclin-1 and Bcl-2 Expression by Intermittent Fasting In CRC: Links to Autophagy and Apoptosis. International Journal of Pharmacy and Biological Sciences. 2025 15 (4): 91-115.
105. K. Pavan Kumar and Amit Kumar. Intermittent Fasting-Induced Autophagy and Apoptosis in Colorectal Carcinoma: The Role of Beclin-1 and Bcl-2. International Journal of Pharmaceutical Biological and Chemical Sciences. 2025 14 (4): 26-38.
106. Kokkula PavanKumar, RajeevImadi, PrasadGarrepally, SNikitha Anthelmintic Activity of Some Medicinal Plants: A Short Review, International Journal of Pharmacy and Biological Sciences,9(2):605-611(2019). (UGC CARE).
107. Kokkula Pavan Kumar, Ampati Srinivas and Prasad Garrepally**,** Hyperthermia has consistently improved the efficacy of radiotherapy and chemotherapy for many types of cancers, Journal of Stem cell Research and Therapeutics International, 1(003):1-3(2019).
108. Kokkula Pavan Kumar, Ampati Srinivas and Prasad Garrepally, Subcutaneous DL technique has proven to be an adequate host for human embryonic stem cells, Journal of Stem cell Research and Therapeutics International, 1(004):1-3(2019).
109. Kokkula Pavan Kumar, Boda Rambabu, Investigational studies on carcinoma in male SD rats, International Journal of Research and Analytical Reviews,6(2):654-662(2019).
110. K. Pavan Kumar, G. Venkateshwarlu, Navaneeth Kumar, Mukesh Sharma, Rekha Pal, Taniya Rawat, E. Rajeswari, Wound healing activity of Indian Medicinal Plants, High Technology Letters, 28(2):1-7(2022).
111. K. Pavan Kumar, G. Venkateshwarlu, Mukesh Chandra Sharma, Govind pal, Rekha pal, Taniya Rawat, Invitro anthelmintic activity of alcoholic and methanolic extracts of seeds of Panicum miliaceum in Indian adult earthworm, High Technology Letters, 28(1): 800-809(2022).

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