Chapter 55

Antimicrobial Activity of Aromatic Herb Essential Oils against *Pseudomonas aeruginosa*

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ABSTRACT

The emergence of multidrug resistance such as *Pseudomonas aeruginosa* is due to the misuse of antibiotics. This has become a significant health concern and calls for the development of novel approaches to combat these challenges. Promising antibacterial activities have been shown by essential oils extracted from aromatic plants, providing a natural and potentially efficient remedy against resistant bacteria. This chapter examines the antibacterial properties of essential oils derived from several aromatic herbs, with a focus on their ability to combat *Pseudomonas aeruginosa*. The chemical makeup of these essential oils, their modes of action, and the effectiveness shown in both in vitro and in vivo investigations are all included in the discussion. Among the important plants that were studied were Sage Oil (*Salvia officinalis*), Thyme (*Thymus vulgaris L.*), Tea Tree Oil (*Melaleuca alternifolia*), Eucalyptus Oil (*Eucalyptus globulus*). The chapter also discusses the implications for clinical applications and the possibility of synergistic effects when conventional antibiotics and essential oils are combined. This chapter intends to contribute to the development of new, natural therapies in the fight against multidrug-resistant *Pseudomonas aeruginosa* by elucidating the antibacterial potential of aromatic plant essential oils.

KEYWORDS
Pseudomonas aeruginosa, Salvia officinalis, Thymus vulgaris L., Melaleuca alternifolia

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INTRODUCTION

P. aeruginosa is a monoflagellated, motile, aerobic, non-spore-forming, rod-shaped, non-fermenting. The genus *Pseudomonas* was initially defined by Migula in 1894, and *P. aeruginosa* was the species type of that genus. *P. aeruginosa* was first isolated from green pus by Gessard in 1882 (Urgancı et al., 2022). Since *P. aeruginosa* is a non-fastidious microbe, it doesn't need any particular growing environment. The majority of non-selective media, such as Mueller-Hinton, Nutrient agar, Luria-Bertani, blood agar, etc., are suitable for its growth; however, some media, such as cetrimide agar and King-A and King-B media, are designed expressly to facilitate the selective propagation of *Pseudomonas*. Although pseudomonads develop best at 37°C, they can withstand temperatures as high as 40°C (Gajdács et al., 2019).

P. aeruginosa has phenotypic traits that include a distinct odor (characterized as flower-like, "grape juice", or "fresh tortilla"), β-hemolysis (on blood agar), and color of the colonies (in the right culture media), which enable rapid organoleptic identification (Clark et al., 2015). *P. aeruginosa* has phenotypic traits that include a distinct odor (characterized as flower-like, "grape juice", or "fresh tortilla"), β-hemolysis (on blood agar), and color of the colonies (in the appropriate culture media), which enable rapid organoleptic identification (Hall et al., 2015; Abbas et al., 2022).

The bacterium is significantly more genetically versatile than other bacteria. It has a relatively large genome, which allows it to grow in a variety of environments, produce a wide range of virulence factors, and exhibit antibiotic resistance to most currently prescribed antibiotics (Cillóniz et al., 20016; Nguyen et al., 2018; Raman et al., 2023). It is a common microorganism found on the surfaces of fruits, vegetables animals, plants, and insects and in water, soil, and sewage (Kaszab et al., 2021). *P. aeruginosa* is mostly responsible for nosocomial infections, including bloodstream, pneumonia, urinary tract infections (UTIs), wounds, and bones and joints (Morin et al., 2021; Sekhi, 2022; Sathe et al., 2023). *P. aeruginosa* is recognized to be linked to lower respiratory tract infections among individuals with cystic fibrosis. It also causes community-acquired infections such as gastrointestinal, skin, soft tissue, and otitis externa (Morin et al., 2021).

Pathogenicity of *Pseudomonas aeruginosa*

Several virulence factors, some of which are essential components of *P. aeruginosa's* cell structure, contribute to the pathogenicity of the bacteria. However, a variety of other virulence factors are also produced and released, contingent upon the conditions surrounding the infection (Azuama et al., 2020; Shaw and Wuest, 2020). The ability of *P. aeruginosa* to adapt to a variety of natural environments and harsh (in vivo) conditions is one of its most essential traits, which is consistent with the genus's ideal metabolic diversity (Chauhan et al., 2023: Abdulhaq et al., 2020).

The primary pathogen-produced virulence factors and hallmark abilities are as follows: (1) biofilm formation factors; (2) iron acquisition systems and factors controlling iron homeostasis (3) extracellular invasive enzymes and secreted toxins; (4) toxic secondary metabolites; and (5) bacterial motility and attachment factors (Chadha et al., 2022).

Table 1: Virulence factor of *P. aeruginosa* and their mechanism in bacterial pathogenesis

Category	Factor	Description/Role
Toxic Secondary Metabolites	Pyocyanin	Generates reactive oxygen species (ROS), induces apoptosis in neutrophils
	Hydrogen Cyanide	Inhibits aerobic respiration, binds to host metalloproteins
Extracellular Invasive Enzymes and Toxins	Exotoxin U (ExoU)	Potent phospholipase causes rapid cell death
	ExoS, ExoT	Disrupt the actin cytoskeleton, inhibit phagocytosis and cell migration
	ExoY	Elevates intracellular cAMP, disrupts actin cytoskeleton
	Exotoxin A (ToxA)	Inhibits protein synthesis, induces apoptotic cell death
	LasB (Elastase B)	Breaks down elastin, collagens, and immune components
	LasA (Elastase A)	Degrades staphylococcal peptidoglycan
	Protease IV (PIV/PrpL)	Cleaves fibrinogen, plasminogen, immunoglobulins
	Alkaline Protease (AprA)	Inhibits neutrophil chemotaxis and degrades complement proteins
	Phospholipase C (PlcH)	Hydrolyzes phospholipids, causes hemolysis
	Phospholipase D (PldA, PldB)	Target PI3K/Akt pathway, promote bacterial internalization
	VgrG2b	Targets microtubules, enhances bacterial internalization
	TplE (Phospholipase)	Induces endoplasmic reticulum stress and autophagy
Motility and Attachment Factors	Type IV Pili	Includes type IVa (involved in twitching motility, retractile due to PilT ATPase) and type IVb (non-retractile, aids attachment to surfaces) pili
	Flagellum	Drives swimming motility in liquid and swarming motility on semi-solid surfaces
	CUP Pili	Five systems (CupA to CupE) crucial for attachment to host surfaces
	BapA, LecA, LecB	Facilitate attachment to solid surfaces and host cells
Biofilm Formation Factors	Exopolysaccharides (Alginate, Pel, Psl)	Critical components of biofilm matrix, contribute to mucoid phenotype
	Extracellular DNA (eDNA)	Integral to biofilm structure
	Rhamnolipids	Aid in biofilm formation, maintenance, and dispersion; provide protection from phagocytes
	Glycine Betaine	Enhances resistance to osmotic stress and survival during lung infections
Iron Acquisition Systems and Factors	Pyoverdine	High-affinity siderophore, sequesters iron from host proteins
	Pyochelin	Functions with pyocyanin, induces oxidative stress
	Heme Acquisition Systems (Phu, Has, Hxu)	Transport heme into periplasm, activate virulence
	Bacterioferritins (BfrA, BfrB)	Store excess iron, preventing toxicity
	Superoxide Dismutase (SodB), Catalase (KatA)	Protect against oxidative damage from excess iron
	Small RNAs (PrrF1, PrrF2)	Regulate response to iron toxicity, critical during infection

Motility and Attachment Factors

The flagellum and type IV pili are essential for *P. aeruginosa* movement. These appendages regulate the bacterium's movement (Table 1) and play a key role in several virulence processes, such as finding a favorable surface and permitting cell-surface contact, changing from a planktonic to a sessile lifestyle, and encouraging the development of biofilms (Geiger and O'Toole, 2023). The bacterial cell is propelled by hydrodynamic forces through the utilization of polar protein complexes called flagella, which enable swimming and swarming motility on semi-solid surfaces. Type IV pili are classified into two primary subfamilies: type IVa and type IVb. Type IVa pili are responsible for driving twisting motility on solid surfaces, and they work in tandem with flagella to produce swarming motility (Geiger et al., 2024). Because of the activity

of PilT ATPase, type IVa pili, which comprises several PilA pilin subunits, are retractile (Little et al., 2024). Flp pilin subunits are the building blocks of type IVb pili, better known as tight adherence (Tad) pili. Since tad pili are non-retractile, they primarily help *P. aeruginosa* adhere to abiotic and biological surfaces. This process is essential for the appropriate colonization and invasion of host tissues as well as the production of biofilms (Webster et al., 2022). The primary adhesins, also known as chaperone-usher pathway (CUP) pili, are essential to *P. aeruginosa* adhesion to host surfaces. Five distinct CUP systems are produced by this bacterium (from CupA to CupE) (Böhning et al., 2023). Additional proteins required for *P. aeruginosa* adherence to host cells and attachment to solid surfaces include the lectins LecA and LecB and the biofilm-associated protein BapA (Böhning et al., 2023).

Biofilm Formation Factors

The development of persistent infections is largely dependent on *P. aeruginosa's* capacity to form a biofilm. Although other species in the host are expected to contribute to *P. aeruginosa's* ability to form biofilms, the majority of experimental data on virulence factors and their regulation came from studies of biofilms from *P. aeruginosa* monocultures (Fernández-Billón et al., 2023). Due to its ability to produce the clinically significant mucoid phenotype when this EPS is overproduced, alginate is regarded as a significant virulence factor of *P. aeruginosa* (Chung et al., 2023). Mucoid cells develop into compact microcolonies that provide defense against immune system attacks and drugs, allowing *P. aeruginosa* to persist over an extended period of time in chronic infections (Muggeo et al., 2023). Additionally involved in *P. aeruginosa* biofilm production at various phases are extracellular secondary metabolites called rhamnolipids. These comprise the initial interactions between cells and surfaces in addition to the upkeep and diffusion of the biofilm structure (Ragno, 2024). Rhamnolipids are considered important virulence factors because they operate as protective agents against phagocytes in biofilms and because they promote the active dispersal of cells from biofilms, hence promoting the colonization of new niches (Skariyachan et al., 2018). Furthermore, when integrated into host membranes, rhamnolipids' amphiphilic qualities might cause host cell tight junctions to break (Wargo, 2013).

Extracellular Invasive Enzymes and Toxins

Extracellular invasive enzymes are essential because they degrade a variety of host connective tissues and immune components early in *P. aeruginosa* colonization of host tissue. Extracellular proteases play a crucial role in the pathogenicity of this bacteria. Examples include protease IV (PIV, also known as lysyl endopeptidase PrpL), elastase A (LasA, staphylolysin), elastase B (LasB, pseudolysin), and alkaline protease (AprA, aeruginolysin) (Galdino et al., 2017). LasB displays elastinolytic activity on human elastin and degrades collagens and different components of the innate and adaptive immune defense, including interferon-γ (IFN-γ), interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), and the surfactant proteins A and D (SP-A and SP-D), which are essential for the phagocytosis of pathogens (Sánchez-Jiménez et al., 2023). In addition to increasing phagocytic evasion, the alkaline protease AprA also inhibits neutrophil chemotaxis and degrades complement proteins and cytokines, such as IFN-α, TNF-γ, and IL-6. *P. aeruginosa* invasion and tissue damage are facilitated by the PIV/PrpL protease's cleavage of fibrinogen. This protease can also hydrolyze plasminogen, complement proteins, immunoglobulin, and host antimicrobial peptides. The proteases LasA, LasB, and PIV/PrpL are secreted to the extracellular media by the *P. aeruginosa* Xcp type II secretion system (Xcp-T2SS), while AprA is secreted by the type I secretion system (T1SS) (Galdino et al., 2017).

P. aeruginosa produces phospholipases, also known as lipolytic esterases, which are an important class of extracellular invasive enzymes. The pathogen uses these enzymes, among other things, to hydrolyze eukaryotic phospholipids (Table 1). This includes the phospholipase C PlcH released by Xcp-T2SS, which has a predilection for phospholipids that include choline, such as phosphatidylcholine and sphingomyelin (Ejike et al., 2023). Phosphocholine and ceramide, which are produced by hydrolyzing sphingomyelin and phosphatidylcholine, respectively, are involved in significant signal transduction cascades in mammalian cells (i.e., growth, differentiation, death, proliferation, and inflammation). Additionally, PlcH produces hemolysis in erythrocytes and humans due to its hemolytic action. The ceramidase CerN expressed in the plcH gene cluster increases its activity (Sánchez-Jiménez et al., 2023). Significantly, PlcH suppresses angiogenesis, or the growth of new blood vessels, and is extremely deadly to endothelial cells.

P. aeruginosa produces toxins and enzymes that cause cytotoxicity and other types of cell death in target host cells, which is one of its main pathogenicity strategies (Wood et al., 2023). Strong cytotoxic A2 phospholipase ExoU (for extotoxin U) can rapidly kill eukaryotic cells by inducing necrosis and a loss of plasma membrane integrity (Muggeo et al., 2023; Song et al., 2023). ExoU killing appears to target epithelial barriers and phagocytes, which encourages bacterial persistence and spread. ExoU is released by *P. aeruginosa* via the type III secretion system (T3SS), a needle complex also referred to as the injectosome that injects toxic effectors directly into the cytoplasm of host cells (Gil-Gil et al., 2023). ExoS, ExoT, and ExoY are among the several toxins that T3SS releases. The bifunctional proteins ExoS and ExoT are composed of an N-terminal GTPase-activating protein (GAP) domain and a C-terminal ADP-ribosyltransferase (ADPRT) domain (deWeever, 2023). By preventing phagocytosis and rupturing epithelial barriers that encourage bacterial dispersion, ExoT ADPRT domain activity suppresses cell motility, adhesion, and proliferation. It targets a more limited and particular subset of host proteins. It has long been believed that ExoS's sole roles are cytotoxic and phagocytic, however new research has revealed that this toxin also encourages *P. aeruginosa* internalization into eukaryotic cells and preserves the intracellular niche (Kroken et al., 2022). The last T3SS-secreted toxin that is known to be involved in infection and host colonization is

ExoY, an adenylate cyclase that increases the intracellular cAMP content in eukaryotic cells and induces the differential regulation of many cAMP-regulated genes. The action of this enzyme results in disruption of the actin cytoskeleton, inhibition of bacterial absorption into host cells, and enhancement of endothelial permeability.

Pseudomonas aeruginosa produces exotoxins and exoenzymes that are injected into eukaryotic cells using a variety of secretion systems, including the type VI secretion system (T6SS). In particular, the phospholipase D enzymes PldA and PldB, which are released by H2- and H3-T6SS, respectively, target the host PI3K (phosphoinositide 3-kinase)/Akt pathway. This allows *P. aeruginosa* to internalize into epithelial cells (Sana et al., 2016). Furthermore, P. aeruginosa produces lipoproteins that have the potential to act as pro-inflammatory lipotoxins, which could cause CF patients' lungs to react to inflammation more exaggeratedly. Examples of this include the osmoprotective lipoprotein OsmE and the lipoaccharide transport proteins A and B (LptA and LptB, respectively), which increase the manufacture of interleukin-8 in human macrophages and host epithelial tissues.

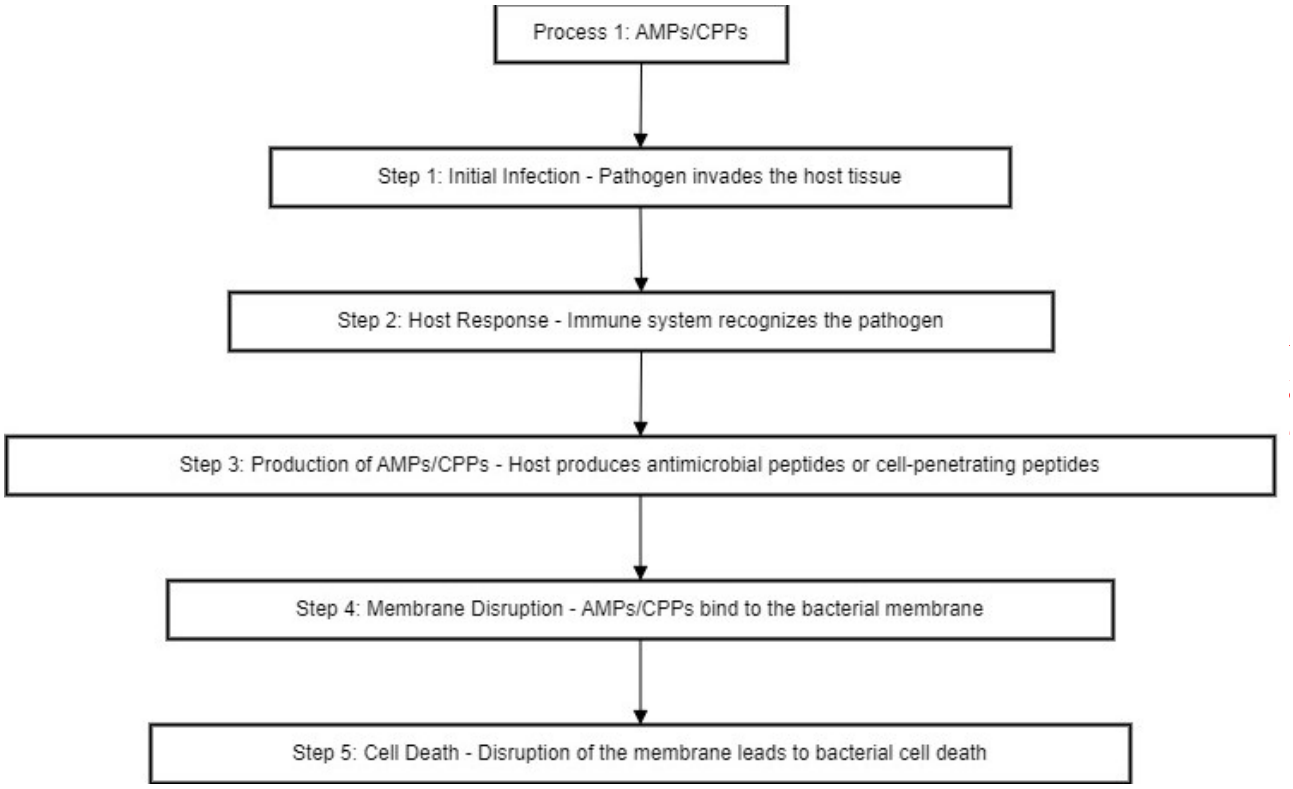

Fig. 1: Pathogenesis of AMPs/CPPs: Initial Infection: Pathogen invades the host tissue.Host Response: Immune system recognizes the pathogen. Production of AMPs/CPPs: Host produces antimicrobial peptides or cell-penetrating peptides. Membrane Disruption: AMPs/CPPs bind to the bacterial membrane. Cell Death: Disruption of the membrane leads to bacterial cell death.

Toxic Secondary Metabolites

Additionally, *P. aeruginosa* produces harmful secondary metabolites that cause cytotoxicity in host cells (Table 1). Phenazines are redox-active chemicals that are produced by this pathogen and are well-known for contributing significantly to both virulence and antibiotic resistance. Particularly noteworthy is pyocyanin, a pigment generated from phenazine that can cause neutrophils to undergo apoptosis by producing reactive oxygen species (ROS) that harm mitochondria (Neve et al., 2024). In addition, *P. aeruginosa* develops hydrogen cyanide, particularly in low-oxygen environments and at high cell densities. By attaching itself to the Fe3+ of the respiratory chain's cytochrome oxidase, this hazardous metabolite prevents the host cells from engaging in aerobic respiration (Besse et al., 2023). Furthermore, cyanide can attach to other metalloproteins due to its structural resemblance to oxygen, which can hinder various cell processes.

Iron Acquisition Systems

All living organisms require iron as a redox cofactor of enzymes for numerous vital processes. The main ways that human iron is kept in cells are either in ferritin or in hemoproteins when it combines with heme. The body uses 'nutritional immunity' during infection to restrict the amount of iron available to pathogens by sequestering it and increasing the production of iron-scavenging molecules such as hemopexin and haptoglobin (Ullah and Lang, 2023). *Pseudomonas aeruginosa* produces the HasAp hemophore and the siderophores pyoverdine and pyochelin, which chelate iron and heme, respectively (Otero-Asman et al., 2019). Its three heme acquisition systems are called Phu, Has, and Hxu. Heme is

transported into the periplasm by TonB-dependent transporters (TBDTs) such as PhuR and HasR, but HxuA, albeit having a lesser function, triggers virulence when it detects heme. Pyoverdine gives the pathogen an advantage during infection because of its strong affinity for iron, which enables it to outcompete host proteins including lactoferrin and transferrin. Pyoverdine-defective *P. aeruginosa* strains exhibit decreased virulence in animal models, which is indicative of this (Gi et al., 2015) While less effective in binding iron, pyochelin is more cost-effective to make and is the recommended option in situations with mild iron deficiency. It causes persistent tissue damage by inducing oxidative stress and inflammation in conjunction with pyocyanin.

Pyoverdine gives the pathogen an advantage during infection because of its strong affinity for iron, which enables it to outcompete host proteins including lactoferrin and transferrin (Kümmerli et al., 2023). While less effective in binding iron, pyochelin is more cost-effective to make and is the recommended option in situations with mild iron deficiency. It causes persistent tissue damage by inducing oxidative stress and inflammation in conjunction with pyocyanin (Kümmerli et al., 2023). The bacterium uses catalase (KatA) and superoxide dismutase (SodB) to reduce oxidative stress and bacterioferritins (BfrA and BfrB) to store excess iron (Lin et al., 2023).

Clinical Relevance of *P. aeruginosa*

P. aeruginosa can spread, avoid immune reactions from the host and harmful antibiotics, create toxins and exoenzymes that harm host cells, and effectively adapt to any environment (Sanya et al., 2023). *P. aeruginosa* is the most common source of infections when considering the global epidemiological aspects of NFGNB (Pourcel et al., 2020). Despite having several virulence characteristics, pseudomonads are not regarded as extremely pathogenic when compared to other bacteria, such as *Streptococcus pyogenes* or members of the Enterobacterales order (Rasoo et al., 2016). Still, they might be accountable for a variety of disease presentations, and these diseases frequently show up as persistent, difficult-to-cure infections. *P. aeruginosa* frequently develops multisite infections as well. The majority of those impacted are immunocompromised patients (Kreitmann et al., 2024). Since it is frequently found in intensive care units (ICUs) and surgical theaters where the widespread use of antimicrobials has allowed for the selection of these microorganisms *P. aeruginosa* is primarily thought to be an opportunistic, nosocomial Gram-negative pathogen, accounting for 13–19% of hospital-acquired infections in the US (Hammoudi Halat and Ayoub Moubareck, 2024; Parcel et al., 2018). Due to *P. aeruginosa's* capacity to survive on a wide variety of inanimate surfaces and to disseminate by aerosol, almost all healthcare facilities have recorded outbreaks and intrahospital infections (Tarafdar et al., 2020). *P. aeruginosa* can only temporarily colonize the digestive tract under normal conditions (however immunocompromised patients may experience an increase in this rate). However, 8–20% of nosocomial infections and outbreaks are linked to people who have colonized the area. Strict adherence to environmental cleaning plans, hand hygiene practices, and infection control measures is essential to preventing nosocomial outbreaks. It's also crucial to identify and eradicate potential infection reservoirs. Clinical presentations may include pneumonia (primarily ventilator-associated pneumonia, or VAP; 10–30%), skin and soft tissue infections related to burns and surgeries (8–10%), "hot tub" folliculitis, "swimmer's ear" otitis externa, eye infections (keratitis), urinary tract infections (UTI; 813.8615%), endocarditis, and bacteremia/sepsis (often secondary to pneumonia or often associated with central line-associated) (Newman et al., 2017; Moradali et al., 2017). *P. aeruginosa* is one of the bacterial pathogens that cause contact lens-associated keratitis. It can cause a corneal ulcer, which is the worst clinical presentation and can occur in 40–60% of cases. This can lead to poor outcomes, fulminant destruction of the cornea, and the loss of eyesight (Khan et al., 2020). The mortality rate from pseudomonad infections, which ranges from 25–39% for pneumonia and 18–61% for bacteremia in hospitalized and immunocompromised patients, is a major concern. These rates may reach 40–70% in the case of MDR isolates (Rojas et al., 2019; Zhang et al., 2020). It was also discovered that cigarette smoke stimulated *P. aeruginosa*, which resulted in the formation of a nfxC drug-resistant phenotype (Xu et al., 2020). *P. aeruginosa* is the most prevalent and well-researched pathogen associated with cystic fibrosis. A mucoid phenotype and a very slimy surface are isolated 3-6 months after *P. aeruginosa* strains with a non-mucoid phenotype first settle in the lungs of CF patients (Armbruster et al., 2021). It has been demonstrated that in CF patients, the age at which *P. aeruginosa* positive first occurred plays a significant role in determining the course of the illness. Between the ages of 0 and 5, the pathogen is present in 10–30% of individuals; after the age of 25, it is prevalent in >80% of patients, and these chronic lung infections are seldom totally cleared. One of the most significant contributing causes to CF patients' deadly pulmonary exacerbations is *P. aeruginosa* (Li and Schneider-Futschik, 2023).

Antibiotic Resistance in *P. aeruginosa*

Generally speaking, the treatment of NFGNB-caused infections is a serious therapeutic challenge for clinicians in both community and hospital settings because of the rising number of isolates that are resistant to many antibiotic classes (Zhen et al., 2020). According to the guidelines set forth by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), isolates categorized as multidrug-resistant (MDR), extensively drug-resistant (XDR), and even pan drug-resistant (PDR) or drug-resistant (TDR) are increasingly showing up in clinical settings (Corona et al., 2023). Antibiotic resistance is primarily caused by two factors: the widespread use of antibiotics in inappropriate contexts and pharmaceutical firms' declining interest in participating in antimicrobial research (Gajdács, 2019; Naeem et al., 2021).

A bacterial species' intrinsic resistance to antibiotics is its inherent ability to reduce an antibiotic's effectiveness through innate structural or functional traits (Baran et al., 2023). Pseudomonas aeruginosa has demonstrated a significant

degree of intrinsic resistance to the majority of antibiotics through mechanisms such as limited permeability of the outer membrane, efflux systems that remove drugs from the cell, and the synthesis of enzymes that inactivate antibiotics, such as β-lactamases (Verdial et al., 2023). Antibiotics cannot pass through the bacterial cell membrane of *P. aeruginosa* and reach their intracellular targets because of the bacterial cell membrane's limited permeability (Huang and Li, 2023). Additionally, the cell membrane of this bacteria has many efflux mechanisms that allow it to expel a variety of drugs. These efflux systems are classified into five primary families: multidrug and toxic compound extrusion, small multidrug resistance, major facilitator superfamily, ATP-binding cassette, and resistance–nodulation–division (Santos et al., 2024; Al-Ouqaili, 2018). In addition to giving drug resistance, these efflux pumps have also added to *P. aeruginosa's* pathogenicity. *P. aeruginosa* can release enzymes that break down antibiotics, specifically focusing on medications such as amikacin, netilmicin, gentamicin, penicillin, streptomycin, aztreonam, kanamycin, neomycin, and tobramycin (Elfadadny et al., 2024).

The bacterium also demonstrates methods of adaptive resistance mediated by polysaccharides and biofilms. *P. aeruginosa* cells have developed phenotypic modifications called biofilms and polysaccharides to shield them against antibiotics (Basit et al., 2021; Chung et al., 2023). As the bacteria that forms biofilms and is least susceptible to medications, *P. aeruginosa* is the main cause of chronic lung inflammation and death in individuals with cystic fibrosis. Additionally, this bacterium acquires antibiotic resistance genes through horizontal gene transfer (HGT) from other bacterial species in the environment, enabling it to evolve resistance to new antibiotics (Michaelis and Grohmann, 2023).

Essential Oils as Potential Tools against AMR

Essential oils (EOs), alternatively referred to as volatile oils, are intricate blends of several volatile, lipophilic chemicals in varying quantities (Yu et al., 2020). Usually, two or three primary components are found in relatively high concentrations (20–70%), with trace levels of additional chemicals present (Xu et al., 2023). Terpenes, which are primarily composed of isoprene units and frequently possess multiple chemical functions like alcohol, phenol, aldehyde, ketone, ether, and hydrocarbon groups, are the major components of essential oils (EOs) (Guimarães et al., 2019). Essential oils (EOs) are secondary metabolites that aromatic plants make to fend against microbes, predators, and harsh weather (Tokgöz, 2024). EOs can be produced by many different portions of the plant and subsequently extracted using a variety of techniques, including solvent extraction (solvent, subcritical water, supercritical CO2), distillation (hydrodistillation, steam distillation, hydro diffusion), solvent-free microwave extraction, and combination techniques (Mahizan et al., 2019). EOs have been shown to have antibacterial, antioxidant, anti-inflammatory, analgesic, antiemetic, and cancer chemoprotective properties in medicinal practice (Man et al., 2019). Additionally, several EOs can be cytotoxic (to kill bacteria, viruses, fungus, protozoa, parasites, and mites), allelopathic, insect repellant, and insecticidal, making them potential substitutes in a range of sectors (Romanescu et al., 2023).

Certain EOs work well against infections that are important to public health. Several EOs have demonstrated in vitro antibacterial action against pathogens on the WHO Priority 1 list (Badescu et al., 2022). Pharmaceutical formulation advances have made it possible to put EOs onto carriers like nanoparticles, greatly enhancing their stability and bioavailability (Nair et al., 2022). But by using spices, the general public can also gain from the antimicrobial EOs' benefits (Li et al., 2022).

Generally speaking, phenols and aldehydes have chemical functionalities that cause antibacterial action, whereas a large percentage of esters, ketones, and terpene hydrocarbons have little to no impact (El-Tarabily et al., 2021). EOs can stop the growth of bacteria by causing membrane proteins to break down and increase cell permeability since they are hydrophobic (Ortega-Ramirez et al., 2020). In many different types of microbes, they can disrupt the expression of genes that code for efflux pumps (*tetA, tetK, pmrA, norA*, *blaTEM*, *blaOXA-23*) (Evangelista et al., 2022). EOs have the potential to impact proton pumps as well, leading to ATP depletion and a decrease in membrane potential. Additionally, EOs can interfere with quorum sensing and prevent the production of biofilms. As a result, they affect gene expression regulation and cell-to-cell communication, two processes that are essential for adaptation in harsh environments (Gurkok and Sezen, 2023). The dilution method (using agar or liquid broth) and the agar diffusion method (using a paper disc or well) are the two most popular in vitro methods for evaluating the antibacterial activity of Eos (Abdollahzadeh et al., 2021).

Aromatic Herbs and their Essential Oils

Aromatic plants' secondary metabolism produces perfumed liquids known as essential oils. Since they are the most important part of the plant, they are referred to as "essential". EOs are concoctions of organic materials made from a variety of plant sources; they give plants their unique scent (Zhang and Piao, 2023). Aromatic herbs are used to extract essential oils (EOs) from a variety of organs, including seeds (caraway, cumin, and coriander), leaves (mint, thyme, sage, rosemary, oregano, basil, celery, and parsley), fruits (anise, fennel, and lemon), flowers (rose and rosemary), bark (cinnamon), cloves or buds (clove and garlic), and rhizomes (ginger) (Mohamed and Alotaibi, 2023). Aromatic plants can produce essential oils (EOs) by combining various organic constituents in the cytoplasm and plastids of plant cells. These pathways include mevalonic acid, malonic acid, and methyl-D-erythrol-4-phosphate (MEP). The resulting compounds are then stored in epidermal cells, secreting fissures, glandular trichomes, or resin canals (da Silva et al., 2021). Depending on the origins of the plants, species, and organs, essential oils have a distinct color and smell. While certain EOs, such green European valerian and blue chamomile, have vivid colors, most are pale yellow or colorless (Ailli et al., 2023). The volatile molecules found in Eos have a crucial role in the ecology because they can shield plants from invading fungi, bacteria, viruses, and insects while also drawing specific insects needed for pollination (Weisany et al., 2024).

Extraction of essential oils

Plant EOs are analyzed using two basic processes: chemical analysis, which takes several minutes, and oil extraction/distillation, which takes several hours (Ashaq et al., 2024). The traditional method used to extract essential oils (EOs) in laboratories is the Clevenger system hydro-distillation, due to the volatile nature of EOs. Although the traditional method used to purify EOs in commercial processes is steam distillation (Karimkhani etal.,20241). Solvent-based EO extraction is widely used in industrial processes, however it is prohibited in the food industry due to the extremely hazardous nature of the organic solvents used (Ghenabzia et al., 2023). To increase the effectiveness, sustainability, and economy of the applicable system, various other techniques have been investigated for the extraction of essential oils (EOs). These techniques include ohmic hydro-distillation and microwave and ultrasonic assisted extraction (Cavallaro et al., 2023). The most important component in ensuring the quality of essential oils is the extraction technique employed, since poor techniques can modify the chemical makeup of aromatic oils, changing both their quality and functionality (Katekar et al., 2023). Additionally, if EOs are extracted using the steam distillation method, the resultant chemicals will always be volatile; but, if solvents are used, the chemical makeup of the extracted EOs will change from that of an equivalent essential oil that is extracted using distillation (Ayub et al., 2023). Selecting the appropriate extraction method based on the properties of each plant material is crucial since the extraction method employed affects the chemical composition of any oil. To preserve consistency in chemical composition, quality, and quantity, the annual extraction of essential oils (EOs) should be carried out under identical conditions each year. These conditions should include employing similar plant parts, a similar extraction technique, and a similar harvesting season. Flowers must be chosen fresh, although plant parts that are collected for extraction can be partially dried, dehydrated, or picked fresh (Saleh et al., 2023).

Biological Activities of Essential oils

Many aromatic plants have been used for centuries in the food business as flavorings and preservatives, as well as as key sources of scent and flavor. Various aromatic plants have medicinal effects, and these effects are mostly caused by EOs (Bolouri et al., 2022). The diverse biological effects of essential oils (EOs) are ascribed not only to their primary constituents, which comprise two or three compounds present in high concentrations, but also to the potent synergistic effects of additional active chemicals. Essential oils are generally used in pharmaceutics for aromatherapy and to enhance the olfactory qualities of pharmaceutical drugs. EOs are used by various traditional systems around the world to treat a wide range of medical issues (Butnariu, 2021). For instance, the essential oils of eucalyptus and clove can treat coughing and bronchitis, respectively, while the essential oils of sage and peppermint can stop the growth of certain bacteria, relieve respiratory congestion, and are well-known carminatives (Kumar, 2016).

Essential oils as Antioxidants

Plants with aromatic properties have phenolic chemicals in their structure, which contribute to their antioxidant action. Among these, flavonoids, phenolic acids, and phenolic terpenes are the most prevalent (Pinto et al., 2021). The removal of free radicals, the formation of compounds with metal ions (metal chelation), and the prevention or reduction of singlet oxygen formation are some of the mechanisms behind the antioxidant action of phenolic compounds. In order to stop free radicals from oxidizing lipids and other biological components, the compounds can supply the hydrogen found in hydroxyl groups in their aromatic rings. Plants primarily include flavonoids and other phenolic chemicals in their leaves, flowers, and woody sections. This is the reason aromatic herbs are frequently employed as remedies, either as essential oil extracts made through processes like distillation and extraction, or as dried leaf and flower parts. The antioxidant properties of aromatic plants differ due to a multitude of factors influencing their chemical makeup (Konfo et al., 2023).

Essential oils as Antibacterial Agents

One significant issue in antimicrobial chemotherapy is the increasing prevalence of antibiotic resistance, which results in inadequate antimicrobial treatment. Antibiotic usage and the ensuing pressure of antibiotic selection are thought to be the primary factors leading to the emergence of various forms of resistant bacteria (Rathore et al., 2023). Plant bioactives, such as EOs, demonstrated potent antibacterial properties against a range of Gram-positive and Gram-negative bacteria (Amin et al., 2023). Several EOs and their major compounds gained widespread recognition due to their strong antibacterial properties. These compounds can be employed as a variety of helpful additives to lengthen food products' shelf lives and ensure the microbiological safety of consumers (Rasheed et al., 2024). Strong antibacterial properties of many EOs have been demonstrated; however, these properties are frequently influenced by the concentration and presence of certain EO constituents, such as terpenoids, alcohols, phenols, ketones, and esters, as well as phenylpropanoids (Zhang and Piao, 2023). The ability of the hydrophobic elements in EOs to interact with the lipids in the cell membranes of microorganisms caused damage to the permeability and solidity of the membrane, which in turn caused high fluctuations in the chain of electron transport, in nutrient uptake, and in the synthesis of nucleic acids and proteins. This, in turn, caused clotting of the cellular contents and inhibited different metabolite enzymes, which ultimately lead to cell death (Bhavaniramya et al., 2019). Following the rupture of bacterial membranes, the bioactive components of EOs can also kill bacteria by penetrating the cell and blocking polysaccharides, RNA, DNA, or proteins (de Sousa et al., 2023). Strong antibacterial properties were demonstrated by several essential oils, such as rosewood, clove, cinnamon, oregano, and lemongrass (Mohamed and Alotaibi, 2023).

Essential oils as Antifungal Agents

Various essential oils and aromatic herbs demonstrated strong antifungal properties against a variety of pathogenic fungi, including yeasts. The target infection and the oil used determine how effective EOs are against it (Corrêa et al., 2023). Effective antifungal activities were demonstrated by the volatile oils of fennel, coriander, and anise against *Candida albicans* at varying concentrations of 1%, 0.5%, and 0.25%, respectively (Hleba et al., 2024). Moreover, lavandula multifida volatile oil demonstrated strong antifungal activity against *Candida albicans* (Alves-Silva et al., 2023). In contrast to *C. albicans* drug-resistant biofilms, several EOs and their constituents exhibited the strongest antifungal effects by inhibiting membrane ergosterol and changing the signaling pathways that stop the yeast from hyphal growth (Augostine, 2023).

Different Important Medicinal Plants Produce Essential oils

For thousands of years, Asian and African civilizations have used medicinal herbs in their traditional medicines. Over the past few decades, there has been a notable rise in public interest in and acceptance of natural medicines in developed as well as developing countries. Approximately 4 billion people worldwide, or 85% of the total population, use herbal medications in place of conventional ones. Additionally, about 25% of all contemporary medications are derived directly or indirectly from medicinal plants (Bhoi et al., 2023).

Research on synthetic pharmaceutical substances indicates that the therapeutic properties of essential oils (EOs) extracted from aromatic and medicinal plants have a wide range of applications. Farmers and researchers have therefore been motivated to develop these chemicals (Swamy and Sinniah, 2016). Medicinal plants that produce EOs are usually found in warm regions.

Of the 3000 recognized essential oils (EOs) produced by different genera of plants, only about 300 exhibit significant commercial potential. Many plants belonging to different families, including the Alliaceae, Lamiaceae, Myrtaceae, Apiaceae, Asteraceae, Rutaceae, and Poaceae families, are capable of synthesizing significant percentages of essential oils and producing them in commercial quantities. These commercial concentrations of essential oils are used in a variety of industries, primarily in the food, pharmaceutical, cosmetic, aromatherapy, agronomy, and polishing sectors (Yingngam, 2023). The most commonly used essential oils with therapeutic value include thyme, oregano, tea tree, eucalyptus, rosemary, lavender, orange, basil, maize mint, lemon, camphor, citronella, eucalyptus, clove, and eucalyptus.

Mechanisms of Antimicrobial Action

Essential oils' antimicrobial properties have been extensively documented. It's still unknown, though, how these essential oils work. Historically, biochemical tests have been the primary means of determining the mode of action of essential oils (Russo and Palla, 2023). These tests are typically not very good at pinpointing the precise causes or pathways that lead to antimicrobial effects because of technical constraints. However, it is thought that an essential oil affects the structure of the bacterial membrane and its transport mechanism.

Membrane Disruption

The modulation of cellular osmotic pressure and the influx and efflux of biomolecules are significant functions of the bacterial membrane (Foster et al., 2024). Therefore, osmotic pressure disruption caused by a weakened membrane will result in intracellular leaking and ultimately cell destruction. The capacity of essential oil to damage bacterial membranes is one of the primary hypothesized mechanisms of action (Angane et al., 2022). The antibacterial activity of EOs is often related to their hydrophobic character, which can have toxic effects on membrane integrity and function (Yap et al., 2021). Research has shown that the EOs' mechanism of action is not independent, but rather linked to a sequence of processes involving the cytoplasm and the cell's outer envelope. Peptidoglycans are present in the cell walls of both Gram-positive and Gram-negative bacteria, and they are crucial for structural integrity and preserving the shape of the cell (Tavares et al., 2020). The phospholipid bilayer that makes up the bacterial plasma membrane serves as a barrier to molecules entering and leaving the cell, which is a general function of the membrane. The thin peptidoglycan and lipid-rich outer membrane of Gram-negative bacteria distinguish them from their Gram-positive counterparts (Garde et al., 2021). Gram-negative bacteria's outer membrane is mostly composed of lipopolysaccharide (LPS), which when combined with porins to form an assembly creates a selective barrier that shields the bacteria from various antibiotics, detergents, and dyes that would otherwise harm the inner membrane (Pandeya et al., 2021). Numerous investigations have revealed that the outer membrane of Gram-negative bacteria functions as an efficient and dynamic barrier to antibiotics (May and Grabowicz, 2018). Consequently, colistin, an antibiotic that targets the outer membrane, is inevitably the last line of treatment for infections brought on by Gram-negative pathogens that are highly resistant to multiple drugs. Nevertheless, EOs offer a viable substitute since they have been demonstrated to have strong effects on both Gram-positive and Gram-negative bacteria in addition to being specific to a certain type of bacteria.

Quantitative measures of potassium leakage, protein leakage, genetic material (DNA and RNA) leakage, and membrane potential are among the quantitative indications of increasing membrane permeability that ultimately result in the loss of cell viability (Khater et al., 2020). High phenolic levels of essential oils (EOs)—such as those containing thymol, eugenol, and carvacrol—were discovered to be the primary causes of the cytoplasmic membrane's breakdown, which allowed protons and other ions to passively flow through (Perumal et al., 2022). Bacterial metabolism heavily depends on the differential between the external and intracellular potentials of the organisms, which is referred to as membrane

potential. The cell membrane's structural damage could be the cause of the drop in membrane potential. The ratio of fluorescent dye intensities inside to outside the cell can be used to measure the potential of the bacterial membrane (Benfield and Henriques, 2020). Following exposure to EOs, depolarized bacterial cells are frequently stained using the membrane potential-sensitive dyes 3,3′-dipropylthiacarbocyanide iodide (DiSC35) and bis-oxonol (Yap et al., 2021). However, it should be noted that modified membrane depolarization may not always result in cell death; rather, it may depend on the extent of modification or whether the cell's functionality was impacted.

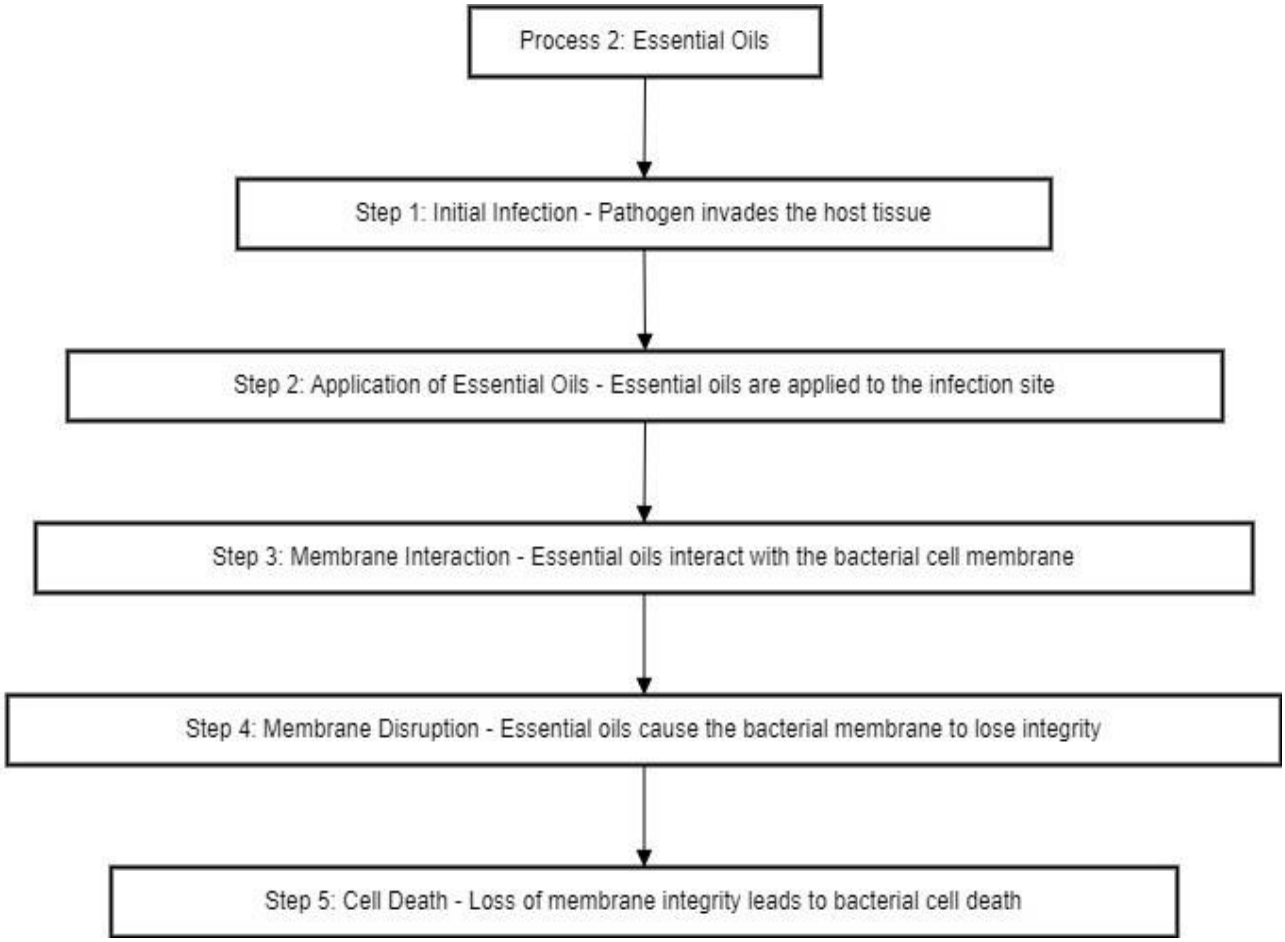

Fig. 2: Pathogenesis of Essential Oils: Initial Infection: Pathogen invades the host tissue. Application of Essential Oils: Essential oils are applied to the infection site. Membrane Interaction: Essential oils interact with the bacterial cell membrane. Membrane Disruption: Essential oils cause the bacterial membrane to lose integrity. Cell Death: Loss of membrane integrity leads to bacterial cell death.

Efflux Inhibition

Impeding the bacterial efflux system is a different perspective on the mechanism of action of essential oil. Specialized channel proteins on the bacterial membrane comprise the bacterial efflux system, necessary for eliminating toxic substances like antibiotics from the intracellular environment (Agreles et al., 2021). Various efflux pumps enable bacteria to thrive in the presence of antibiotics, from compound-specific pumps to general pumps. To combat antibiotic resistance and restore the effectiveness of medicines, it is crucial to inhibit the functioning of these pumps.

Essential oils against *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a well-known bacterium that is resistant to multiple drugs. Essential oils have drawn interest for their possible antibacterial qualities. Research on the efficacy of several essential oils against *Pseudomonas aeruginosa* has been done in vitro.

Tea Tree Oil (Melaleuca alternifolia)

Melaleuca alternifolia leaves are used to make tea tree oil, which is well known for having antibacterial qualities. Its primary active ingredient, terpinen-4-ol, works well against a variety of infections, including *Pseudomonas aeruginosa*, when combined with other terpenes and phenolic chemicals (Haines et al., 2022). Through an increase in fluidity and permeability, terpinen-4-ol damages bacterial cell membranes, causing internal leakage and ultimately cell death. Tea tree oil further suppresses bacterial development by interfering with bacterial enzyme systems, including ATP generation.

According to studies, tea tree oil is useful against persistent infections because it prevents *Pseudomonas aeruginosa* from forming biofilms and can disturb existing biofilms. According to some research, combining tea tree oil with antibiotics like ciprofloxacin or gentamicin may have synergistic effects that boost antibacterial activity. Tea tree oil resistance is possible, although it seems less common than with traditional antibiotics—possibly because of the oil's intricate makeup (Nascimento et al., 2023).

Thyme (Thymus vulgaris L.)

Essential oil (TEO) derived from thyme (Thymus vulgaris L.) is frequently utilized as a substitute treatment for a number of infections, including upper respiratory tract infections (Kowalczyk et al., 2020). TEO has antiviral, antibiofilm, antibacterial, and antifungal qualities as a result of its biological action (Tariq et al., 2019). The chemical makeup of TEO may influence its biological action. Thymol, the main ingredient in EO, has been shown to possess antiviral, antibacterial, antifungal, and antihyperglycemic properties. The antibacterial impact of TEO may be enhanced or altered by its constituents, which include carvacrol, p-cymene, γ-terpinene, β-myrcene, linalool, and terpinen-4-ol (Heghes et al., 2020). The plant's phenophase and the time of oil preparation can change the chemical composition of essential oils (EOs) and hence affect their biological activity (Pandur et al., 2021). Strong antibacterial effects are exhibited by bioactive components including carvacrol and thymol found in thyme oil, which is derived from Thymus vulgaris. Carvacrol and thymol damage bacterial membranes, causing cellular contents to seep out and ultimately bacterial death. *Pseudomonas aeruginosa* has been effectively inhibited in its proliferation and biofilm formation by thyme oil. Combined with drugs such as ceftazidime and ciprofloxacin, it has also shown synergistic effects. To completely comprehend its methods of action against *Pseudomonas aeruginosa* and its possible therapeutic applications, more research is necessary (Ribeiro et al., 2022).

Sage Oil (Salvia officinalis)

Salvia officinalis is a plant that belongs to the mint family. It is angiosperm, a dicotyledonous flower with united petals, in the order Toby Floral, suborder Mangroves, family Mint, and gender Salvia (Pejčić et al., 2020). Evergreen perennial salvia has woody branches, green leaves, and violet-blue blooms. The leaves are gray-green in hue, with creases on their top surface and nearly white, soft fluff on their lower surface. The genus Salvia has some species that have important medicinal properties, such as salvia officinalis. It worked well for relaxation, decreasing blood sugar, and other things. Thujone, 1, 8 cineole, Borneol, Borneol acetate, sesquiterpene, tannins, and phenolic acids are among the compounds found in this plant (Shaaban, 2020). The chemical makeup of sage oil is complex and includes phenolic chemicals, monoterpenes, and sesquiterpenes, which are responsible for its antibacterial properties. It has been demonstrated that these substances have antibacterial properties against a variety of infections, including *Pseudomonas aeruginosa* (Swamy et al., 2016).

Eucalyptus Oil (Eucalyptus globulus)

Along with other terpenes and phenolic compounds, 1,8-cineole, or eucalyptol, is the main ingredient of eucalyptus oil, which is derived from Eucalyptus globulus. By rupturing bacterial membranes, blocking bacterial enzymes, and interfering with cellular functions, 1,8-cineole has antibacterial action (Chandorkar et al., 2021). *Pseudomonas aeruginosa* has been shown to be effectively inhibited by eucalyptus oil, which also decreases the virulence factors like elastase and pyocyanin produced by the bacteria. Because of its broad-spectrum antibacterial activity, it can be used in a variety of clinical settings, such as environmental disinfectants, mouth rinses for dental hygiene, and topical preparations for respiratory infections (Sagar et al., 2022).

Invitro Techniques to Access the Antibacterial Activity

Agar diffusion (using a paper disc or well) and dilution (using agar or liquid broth) are the two most popular in vitro methods for evaluating the antibacterial activity of Eos (Abdollahzadeh et al., 2021). Regarding cost and methodology, agar diffusion methods rank among the most practical approaches. Using the spreading plate approach, a pathogenic bacterium is introduced onto an agar plate in the agar well diffusion method (a precise volume of the microbial solution is dispersed over the surface of agar, through a glass diffuser). The tested solution (such as extract) is poured into a well or hole formed aseptically using a sterile cork borer with a diameter of 6–8 mm, and the area is then incubated at the ideal temperature and humidity. The tested solution will gradually permeate the agar media, stopping the growth of the bacterium. The inhibitory zone's diameter will be assessed afterward. A filter paper disc holding the test solution is placed on the agar medium and inoculated with the tested microorganisms using the agar disc diffusion method (Abdollahzadeh et al., 2021). In general, agar diffusion techniques facilitate the facile testing of several extracts/substances against diverse microorganisms; nonetheless, they cannot elucidate the minimum inhibitory concentration (MIC) or the capacity of a substance/extract to impede or eliminate a merorganization. Using the dilution method, one can precisely count the live cells in a culture of bacteria, fungi, or viruses by creating successive dilutions of concentrated solutions of microbial strains. Every diluted sample is mixed with agar medium that has been liquefied, and then put into a petri dish where it solidifies and contains the bacteria in its matrix. As the germs spread around the agar plate, they can be precisely counted. This technique is used to find a substance's minimum inhibitory concentration (MIC) and its capacity to either kill or stop the growth of the strains under investigation. Additionally, it serves as the benchmark for testing for antibiotic susceptibility. The minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of the chosen AB are

commonly used to characterize bacterial resistance. Nonetheless, there is a great deal of variation in the reported values for MIC and MBC, which may be due to the large variety of bacterial strains, methodological differences, and research design changes (Ayobami et al., 2020). Interestingly, a number of factors that affect the extract's variability (such as the plant component employed and the extraction technique) also affect the antibacterial activity of essential oils.

Synergistic Interactions between the Essential Oil and Antibiotic

The combination of antibiotics with plant extracts affects bacteria that are resistant to them, providing new therapeutic options for diseases caused by pathogens. Synergy therapies have the potential to reduce adverse effects and the active daily dosage of antibacterial agents required for therapy. Combining essential oils with antimicrobials to counteract multidrug-resistant bacteria is one of the newest approaches in this fight. Because new antibiotics aren't coming onto the market and more pathogens are becoming resistant to them, Three distinct outcomes synergistic, additive, or antagonistic can arise from the interaction of antimicrobials in combination. When two antimicrobial chemicals are combined, they produce antibacterial activity that is larger than the sum of their separate antibacterial activity. This is known as synergy. Combining antimicrobials results in an antimicrobial impact that is equal to the sum of the effects of each compound alone, which is known as an additive effect. Two chemicals that operate antagonistically on one another reduce each other's antibacterial activity as compared to when they act separately (Sharma et al., 2020).

A Gram-negative bacterium known for both its quick acquisition of acquired resistance mechanisms and innate resistance to numerous drugs is *Pseudomonas aeruginosa*. Because there are few therapeutic options for *Pseudomonas aeruginosa* infections in clinical settings, these infections present substantial problems. Antibiotics that are frequently used to treat *Pseudomonas aeruginosa* include; Broad-spectrum antibiotics such as carbapenem (Imipenem, meropenem, and doripenem) are frequently used as first-line treatments for severe *Pseudomonas* infections, particularly those that are resistant to other antibiotic classes (Langendonk et al., 2021). They work well against strains of bacteria that are resistant to many drugs and have strong tissue penetration. *P. aeruginosa* is susceptible to the antibacterial properties of cephalosporins, among which ceftazidime and cefepime are fourth- and third-generation antibiotics, respectively. Cephalosporin resistance, particularly to ceftazidime, has increased, nevertheless, as a result of the advent of AmpC and extended-spectrum beta-lactamases (ESBLs). *P. aeruginosa* infections have been treated with fluoroquinolone antibiotics such as levofloxacin and ciprofloxacin. However, chromosomal mutations and efflux pump mechanisms have led to the widespread development of fluoroquinolone resistance. Three aminoglycoside antibiotics that are frequently used in combination therapy to treat *P. aeruginosa* infections include gentamicin, tobramycin, and amikacin. They can aid in overcoming resistance mechanisms and frequently work in concert with beta-lactam antibiotics

It has been suggested that combining essential oils with medicines can improve their antibacterial effectiveness against *Pseudomonas aeruginosa*. Antibiotics and essential oils can work synergistically to promote bacterial susceptibility and overcome resistance mechanisms (Ju et al., 2022). Increased intracellular accumulation of antibiotics, potentiation of antibiotic activity, suppression of resistance mechanisms, and improved permeability of bacterial cell membranes are some of the processes causing these synergistic effects. Carvacrol and ciprofloxacin, thymol and gentamicin, tea tree oil, and imipenem are a few examples of synergistic pairings. These mixtures have shown potential for therapeutic applications by exhibiting increased antibacterial activity against *P. aeruginosa* in vitro. A few examples of synergistic pairings are ciprofloxacin and carvacrol, gentamicin and thymol, and imipenem with tea tree oil. In vitro tests of these combinations have revealed increased antibacterial activity against *P. aeruginosa*, indicating potential for use in clinical settings (Herrera-Espejo et al., 2020).

Practical Applications

The antibacterial properties of essential oils can aid in preventing wound infections. Certain oils are well known for their capacity to heal wounds, including chamomile oil (*Matricaria chamomilla*), tea tree oil (*Melaleuca alternifolia*) and lavender oil (*Lavandula angustifolia*). These oils can be diluted and applied topically to clean wounds to help promote speedier healing and prevent the formation of dangerous bacteria (Gadisa and Usman, 2021).

Essential oils are powerful disinfectants because of their inherent antibacterial qualities. The antimicrobial properties of several oils, including eucalyptus (*Eucalyptus globulus*), lemon (*Citrus limon*), and thyme (*Thymus vulgaris*), have been investigated. They offer a healthy substitute for chemical-based disinfectants on surfaces in households, healthcare facilities, and locations where food is prepared. Research has demonstrated that specific essential oils possess preservation properties, which can aid in prolonging the shelf life of food items. Certain oils, such as those derived from rosemary (*Rosmarinus officinalis*), oregano (*Origanum vulgare*), and cinnamon (*Cinnamomum verum*), has antibacterial qualities that can impede the development of foodborne pathogens and spoilage organisms. By incorporating trace amounts of these oils into food products, chemical preservatives may be avoided by helping to guard against microbial infection and spoiling (Gadisa and Usman, 2021).

Safety and Toxicity Considerations

Since essential oils are quite concentrated, it's best to dilute them before using them to lower the possibility of negative reactions. The oil and the planned use determine the safe dilution ratio. For topical usage, general guidelines advise dilution of essential oils to a concentration of 1–3%in a carrier oil (Hoang et al., 2021). For certain populations, such

as toddlers, pregnant women, or people with sensitive skin, dilution ratios might need to be modified. Individuals may experience negative effects from essential oils, such as sensitization, respiratory problems, allergic reactions, or skin irritation. Before applying any new essential oil, especially on sections of the skin that are sensitive, it is imperative to conduct a patch test to rule out any potential responses (Garg et al., 2021). Certain essential oils have the potential to be harmful if misused or used in significant amounts. Essential oils should not be used internally unless directed by a licensed healthcare professional. Some oils, including eucalyptus and wintergreen (Gaultheria procumbens), have significant concentrations of particular chemical components that are poisonous if consumed. Additionally, as some essential oils can be toxic or hazardous to animals, caution should be used while using them near pets (Bunse et al., 2022).

Conclusion

The study of aromatic plant essential oils' antimicrobial activity against *Pseudomonas aeruginosa* reveals encouraging findings that highlight their potential as strong treatments for bacterial illnesses. A thorough analysis of the literature reveals that these essential oils have significant inhibitory effects against *P. aeruginosa*, a bacterium that is notoriously difficult to treat and resistant to a variety of drugs. critical oils possess a wide range of bioactive components, such as terpenes, phenols, and aldehydes, which play a role in their antibacterial capabilities. These molecules damage bacterial cell membranes, decrease enzyme function, and interfere with critical microbial processes.

The practical applications of essential oils' antimicrobial properties span a wide range of domains, such as food preservation, hygiene, and healthcare. Applying essential oils topically, for example, maybe a safe, effective way to promote wound healing and avoid infections in wound care. Similar to this, essential oils offer effective microbial control without the hazards of toxicity or environmental damage, making them a safer and more ecologically friendly option to synthetic chemical agents in the disinfection space. Furthermore, food safety and quality can be improved in the food business by utilizing the antibacterial and antioxidant qualities of essential oils to prolong the shelf life of perishable food items and prevent the formation of foodborne infections.

Even though essential oils have a lot to offer in terms of antimicrobial applications, it is crucial to use caution and take safety precautions into account when using them. The danger of negative responses must be reduced by following the right dilution and application procedures, especially in people who are allergic to or sensitive to specific plant chemicals. Standardized testing procedures must also be created in order to guarantee correct evaluation of the antimicrobial efficiency of essential oils, making study comparisons easier and boosting the validity of research findings.

Future study should focus on a few key areas to better understand the potential of essential oils as antibacterial agents. While investigation of lesser-known botanical sources may provide novel bioactive chemicals with strong antimicrobial properties, standardization of testing techniques will allow more reliable assessments of antimicrobial activity. Furthermore, studying the synergistic relationships between traditional antibacterial drugs and essential oils may result in the creation of improved formulations with higher efficacy and lower resistance risk.

The study concludes with the noteworthy potential of aromatic herb essential oils as useful tools in the battle against microbiological illnesses, particularly *P. aeruginosa* infections. Essential oils present promising ways to combat antimicrobial resistance, enhance public health outcomes, and promote sustainable practices in food safety, cleanliness, and healthcare by utilizing their natural sources and strong antibacterial qualities. To fully realize the medicinal potential of essential oils and ensure their safe and efficient use in a variety of applications, however, more study must be done as well as careful consideration of safety issues.

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