

NANOPARTICLES ENRICHED WITH ESSENTIAL OILS FOR BIOFILM INFECTION CONTROL

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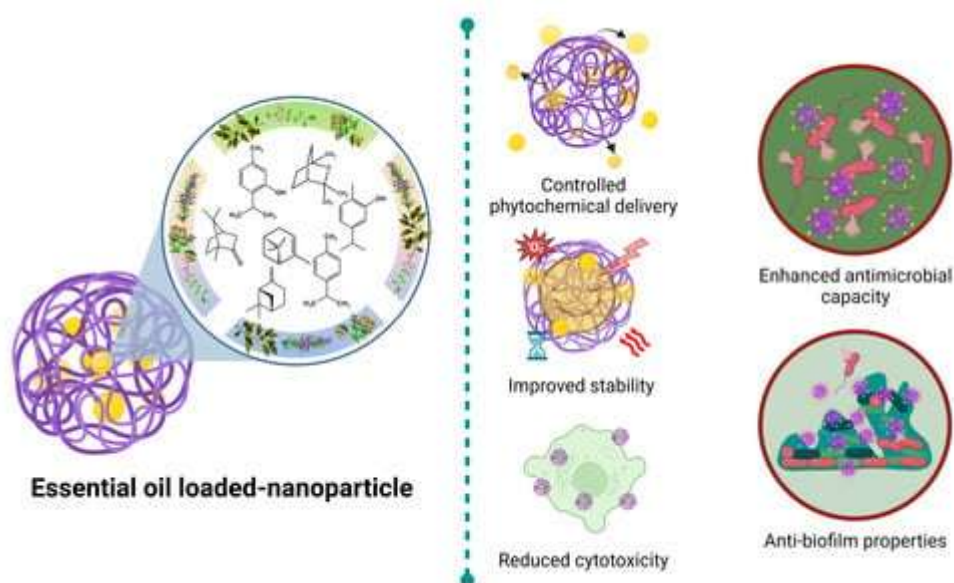
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ABSTRACT

Microbial pathogens are major causes of long-term illnesses and deaths worldwide. While antibiotics have been effective in treating infections, overuse has led to resistance in bacteria, fungi, viruses, and parasites. Additionally, some microbes are naturally resistant, and the body's defenses complicate the treatment of recurrent, stubborn infections or those caused by new diseases. Essential oils, rich in compounds that combat microbes, face challenges in medical use due to their volatility, poor solubility in water, and tendency to react with other chemicals. To address these issues, researchers have explored nanoparticles made from biodegradable polymers as carriers for antimicrobial agents. These nanoparticles can improve the delivery and stability of essential oils, making them more effective in fighting infections. They offer several benefits, including better antimicrobial action, increased oil retention, improved water solubility, and enhanced stability. This paper focuses on the use of nanoparticles to enhance the efficacy of essential oils against microbial infections, particularly those involving biofilms—complex microbial clusters that are hard to treat. By using nanoparticles to deliver essential oils, their effectiveness against infections can be significantly improved, offering a promising alternative to antibiotics in food safety, packaging, and medical treatments.

Keywords: Essential Oil, Nanoparticles, Biofilms, Infections, Antibiofilm



Graphical Abstract

Introduction

Chronic diseases are caused by biofilms, which are structured microbial colonies attached to surfaces and submerged in extracellular polymeric substance (EPS), in both medical and industrial settings. They are notoriously resistant to conventional antimicrobial treatments due to their protective biofilm structure and altered metabolic states. The emergence of multidrug-resistant (MDR) pathogens necessitates alternative strategies [1].

An increasing global health concern took on by the development of antimicrobial agents is antimicrobial resistance in microorganisms like as bacteria, fungi, viruses, and parasites. By 2050, it is expected to cause 10 million deaths and more than two million infections yearly. Through gene transfer techniques including conjugation, transformation, and transduction, resistance spreads. To address this problem, new and inventive antibacterial methods are urgently needed [2, 3].

Essential oils possess a diverse range of antibacterial characteristics and are extracted from medicinal plants. However, their therapeutic usefulness is diminished by their limited absorption, low stability, and excessive volatility [4]. By encasing EOs in nanoparticles to improve their stability, bioavailability, and targeted distribution, recent advancements in nanotechnology present a viable remedy [5].

Plant-based volatile aromatic molecules called essential oils (EOs) have a diverse range of antibacterial qualities. Despite its potential, EOs' poor solubility, volatility, and instability limit their clinical use. Encasing EOs in nanoparticles to improve bioavailability, controlled release, and targeted antibacterial activity has made nanotechnology-based delivery systems a feasible way to get around these restrictions [6, 7].

Essential oils (EOs), volatile aromatic compounds derived from plants, possess broad-spectrum antimicrobial properties. Despite their promise, the clinical use of EOs is restricted by weak solubility, volatility, and instability [8]. To overcome these

limitations, nanotechnology-based delivery systems have emerged as a viable approach, encapsulating EOs in nanoparticles to enhance bioavailability, controlled release, and targeted antimicrobial activity [9].

The biochemical nature of essential oils depends on numerous aspects such as the type of plant, the climate, the soil, the kind of fertilizer used, the plant's genetic makeup, how it's grown, when it's harvested, and the way used to abstract the oil. Essential oils mainly contain two types of chemicals: hydrocarbon terpenes (also called isoprenes) and terpenoids (also known as isoprenoids), along with aromatic and aliphatic compounds [10]. Terpenes are the most common type of chemical found in essential oils. These are made up of five-carbon units called isoprene (C_5H_8). Terpenes can be categorized based on how many carbon atoms they have, such as mono, di, tri, tetra-terpenes, or hemi-terpenes. Among these, monoterpenes and sesquiterpenes are the most significant parts of essential oils and are responsible for their distinct scent. Sesquiterpenes are made up of three isoprene units and can have different structures like acyclic, monocyclic, bicyclic, or tricyclic [11].

This review focuses on multidrug-resistant bacteria, both in their free-floating and biofilm forms. It provides a detailed summary of recent progress in studying chitosan and zein nanoparticles (NPs). These biopolymers were chosen for their potential uses in the biomedical field. The review also includes an in-depth look at alternative treatments for microbes that are resilient to antibiotics and for biofilms that are hard to eliminate. It highlights how different types of biopolymer-based nanocarriers can be used effectively against these challenges. This research shows how nanoparticles can help tackle problems caused by persistent biofilms and antibiotic-resistant bacteria. It also supports the development of more targeted anti-biofilm strategies and helps improve our understanding of how these substances inhibit bacterial growth [12, 13].

Essential Oils as Antimicrobial Agents

Essential oils (EOs) as thymol, eugenol, cinnamon aldehyde, carvacrol have demonstrated potent antibacterial activity by breaking down microbial membranes, denaturing proteins, and disrupting enzyme activity. Their poor solubility and rapid disintegration limit their medicinal utility despite these attributes [14] [15].

Essential oils (EOs) like oregano, clove, cinnamon, and rosemary have received a lot of attention from the scientific community. These essential oils, which are primarily made up of aldehydes, terpenes, and phenols, exhibit a wide range of biological functions, such as antibacterial, antifungal, antiprotozoal, antioxidant, anti-inflammatory, and anticancer properties [16, 17].

Instead of isolating pure components, the use of natural oils enhances the synergistic action of phytochemicals, enabling them to work through multiple phases to increase antimicrobial activity. Cell membranes can be targeted by compounds with functional groups or lipophilic compounds like phenols and terpenes. Substances like eugenol and cinnamon aldehyde can enter the cytoplasm and mitochondria and stop bacteria from producing proteins and ATP, which are necessary for survival, by interacting with proteins and enzymes. Additionally, it has been discovered that components in natural oils disrupt the efflux pump mechanism, which is generated by drug-resistant bacterial strains [18].

Because of their diverse chemical composition, essential oils may be able to fight

biofilm. The anti-biofilm qualities of essential oils may be impacted by the different biofilms that are created by different microorganisms. Bacterial biofilms were effectively broken down by essential oils that contained high concentrations of monoterpenes or phenylpropanoids. When planktonic cells are still involved in the early stages of biofilm formation, these two chemical groups are more active, and as the number of sessile microorganisms keeps growing, their effectiveness decreases [19, 20].

Extraction methods for essential oil

Essential oils can be extracted using a variety of simple and well-liked techniques, such as hydro distillation, steam distillation, and cold pressing. Supercritical fluid and microwave extraction are two other possible extraction methods that have been employed [21]. Plants' bark, seeds, flowers, and roots can all yield essential oils. A number of factors influence the composition of essential oils, including climate, the soil in which the plant is grown, the culture method, age, the terrestrial source, and abstraction processes. Although the different essential oils have different chemical compositions, terpenes, terpenoids, alcohol, ester, and phenol are the main chemical components of essential oils [22].

Conventional extraction methods

Hydro Distillation

Avicenna created the first and most fundamental method of oil extraction, hydrodistillation, and was the first to establish extraction using alembics. Rose was the first plant extract to be used and refined in this way. The process begins by putting the plant parts straight into water inside the alembic, then the whole mix is boiled. The device has a heating source, an alembic container, a condenser that turns the vapor into liquid, and a decanter to gather the liquid and separate the essential oils from the water [8]. This method is considered a new way to extract parts from plants like wood or flowers. It's commonly used for extracting materials from natural plants that don't mix well with water and have a high boiling point. Since the essential oils stay in the water, this method can extract some of them without heating them up. The main benefit of this technique is that it can separate plant parts at temperatures below 100 degrees [21].

The use of hydrodistillation to extract essential plant oils has not received much attention. The production and characteristics of essential oil from rosemary (*Rosmarinus officinalis* L.) were examined by Okoh et al. 2010 in relation to solvent-free microwave extraction (SFME) and hydrodistillation (HD). The yield of the total volatile fraction from SFME was 0.39%, while that from hydrodistillation was 0.31% [23].

Steam distillation

The most popular method for obtaining essential plant oils is steam distillation. This method extracts 93% of the essential oils, with the remaining 7% being extracted further using different techniques [24]. To increase the yields of extracted essential oils while lowering the quantity of undesired water made by the extraction method, Masango developed a novel steam distillation extraction method. Above the steam

source, a dense bed of plant samples is used in this method. Boiling water has no effect on the botanical components; only steam is permitted to flow through the plants. As a result, less steam is used in the process, and less water is needed to produce the distillate [25].

3. Solvent extraction

This method uses common solvents like acetone, petroleum ether, hexane, methanol, or ethanol to take out delicate or fragile flower parts that can't be removed with heat or steam [26].

Commonly, the plant samples are immersed with the solvents to be separate out and heated gently. The solvents are then removed and allowed to evaporate. A resin (resinoid), an alloy of wax, essential oil, and fragrance, is present in the filtrate. After dissolving the essential oil in the filtrate mixture with alcohol, the mixture is then distilled at a low temperature. While the aromatic absolute oil stays in the pot residue during the distillation process, the alcohol absorbs scent and disappears. This approach to essential oil extraction is more expensive and time-consuming than others because of its complexity [27].

Different kinds of essential oils used for biofilm

Cinnamon Oil

Around the world, cinnamon is commonly used in traditional medicine. The bark and leaves of cinnamon have many good properties, such as fighting bacteria, fungi, and inflammation, and they can also help with cancer, pesticides, and blood sugar control. Natural cinnamon oils contain several important chemicals, like trans-cinnamaldehyde (TC), cinnamyl acetate, camphor, caryophyllene oxide, beta-caryophyllene, L-bornyl acetate, eugenol, alpha-cubebene, alpha-terpineol, terpinolene, and alpha-thujene. Even though cinnamon has many health benefits, some types of bacteria, especially gram-negative ones, are harder to kill with natural oils like cinnamon essential oil. This is mainly because these bacteria have porin proteins that allow certain substances to pass through but block others [28].

One of the main parts of cinnamon essential oil is trans-cinnamaldehyde. This type of chemical can get into the wall of bacteria, stick to proteins, and break down how the wall keeps things in and out. Furthermore, it inhibits the movement of proteins and ions, resulting in cytoplasmic coagulation, protein denaturation, and a reduction in metabolites and ions, all of which contribute to the death of bacteria. TC helps make the bacterial membrane stiff by taking up a lot of the fatty acids in the phospholipid membrane. Apart from TC, other components hinder the proper operation of the mitochondria's electron transport chain, which stops the synthesis of ATP. Moreover, it stops the bacterial cell division regulator protein FtsZ from polymerizing in a GTP-dependent manner. The CEO affects the expression of luxR and bcsA, two crucial elements of the quorum sensing cell communication system required for biofilm structuring [29].

Rosemary Oil

Rosemary (*Rosmarinus officinalis* L.), a shrub belonging to the Lamiaceae family, is well known by potent antibacterial and antioxidant qualities as well as its organoleptic qualities. It has a long history in traditional medicine like pulmonary antiseptic,

choloretic, cholagogue, antidiarrheal, antirheumatic, and, antiviral, anti-inflammatory, and anticancer properties. Camphor, linalool, p-cymene, α -pinene, β -pinene, eucalyptol, and γ -terpinene are the constituents of RMEO. Despite a large number of studies on RMEO's antibacterial activity, there is disagreement regarding its mode of action. RMEO works well against Gram-positive bacteria, with minimum inhibitory concentrations (MIC) lies between 0.020 $\mu\text{g/mL}$ to 0.8 $\mu\text{g/mL}$. Certain researchers claim that their antibacterial features come from its high terpene content, which lyses and disarrays cell membranes [30]. Although polyphenols are uncommon, some people believe they have bactericidal properties. By interacting with cell membrane proteins, changing their morphology and function, and interfering with mitochondrial electron transport, polyphenols degenerating genetic material and essential nutrients. Fatty acid synthesis could also be affected. Some researchers argue that the effectiveness of RMEO is due to the synergy between terpenes and polyphenols, regardless of their ratios [31].

Clove Oil

Clove essential oil (CLEO) (*Syzygium aromaticum*) is mostly composed of eugenol, with trace amounts of β -caryophyllene, eugenol acetate, and other components. CLEO has a wide spread properties, including antibacterial, antioxidant, insecticidal, antifungal, anti-inflammatory, antiviral, anticancer, analgesic, antispasmodic, and organoleptic effects [32]

The antibacterial characteristics of CLEO, which are more related to those of eugenol, function similarly to trans-cinnamaldehyde (TC) against Gram-negative bacteria. Eugenol's chemical characteristics make it reliable to enter the cytoplasm and cross the lipopolysaccharide membrane, resulting in the depletion of cellular components. The free -OH group in CLEO's structure aids in its antibacterial activity against Gram-positive bacteria like *P. aeruginosa* [33].

Oregano Oil

Owing to its organoleptic properties, oregano essential oil (OEO) has long been used in medicine to treat indigestion, diarrhea, asthma, cramps, and contraception. Recent research indicates that the components of OEO, which include α -humulene, o-cymene, trans-caryophyllene, carvacrol, thymol, and γ -terpinene, are primarily responsible for its potent antibacterial and antioxidant qualities [34].

Carvacrol, a phenolic molecule, is said to be important even though the accurate antibacterial mode of action of OEO is still unknown. Carvacrol alters the structure and function of the cell membrane, causing metabolic imbalance, disruption of nucleic acid formation or function, expulsion of intracellular parts, and interference with quorum sensing to protect the formation and spread out of biofilms [35].

Carvacrol's ability to penetrate the lipid bilayer destabilizes it, causing the membrane to swell and become more permeable. By lowering the internal pH and the electrochemical membrane potential, this has an impact on ATP synthesis. Experimental evidence indicates, outflow of essential ions for example H^+ and K^+ is necessary for microbial cell apoptosis. By functioning as an ion exchanger, the -OH group in carvacrol encourages cation exchange in bacterial cells. As carvacrol interacts with the membrane and moves through it to enter the cytoplasm, it loses the

acid proton in its uncharged state [36].

Increased amounts of heat shock proteins and non-motile cells are seen in *Escherichia coli* O157:H7 exposed to carvacrol (Table 1). This leads to morphological and physiological changes linked to modifications in the peptidoglycan composition and inhibits the formation of biofilms. By depleting N-acyl homoserine lactone, a signal molecule necessary for the synthesis of enzymes that break down plant cell walls, OEO interrupt with quorum sensing through carvacrol's reaction with ExpI/ExpR proteins [37].

Table 1. Effect of essential oil against biofilm

Essential Oil	Main Active Compounds	Target Microorganisms	Effect on Biofilm Formation	Mechanism of Action
Tea Tree Oil	Terpinen-4-ol, α -terpineol	<i>Staphylococcus aureus</i> , <i>Candida albicans</i>	Decreases biofilm biomass and metabolic action	Disrupts cell membranes; inhibits quorum sensing
Cinnamon Oil	Cinnamaldehyde	<i>E. coli</i> , <i>P. aeruginosa</i>	Inhibits biofilm initiation and disrupts mature biofilms	Alters cell wall integrity; inhibits EPS synthesis
Clove Oil	Eugenol	<i>Staphylococcus epidermidis</i> , <i>Candida</i> spp.	Reduces biofilm viability and thickness	Interferes with membrane permeability and adhesion
Oregano Oil	Carvacrol, Thymol	<i>P. aeruginosa</i> , <i>Listeria monocytogenes</i>	Strong inhibition of biofilm formation and structure	Disrupts bacterial membranes; anti-quorum sensing
Lemongrass Oil	Citral, Geraniol	<i>Salmonella</i> spp., <i>S. aureus</i>	Reduces adhesion and biofilm matrix formation	Inhibits motility and gene expression related to biofilm
Eucalyptus Oil	1,8-Cineole	<i>S. aureus</i> , <i>E. coli</i>	Moderate reduction in biofilm formation	Oxidative stress induction; cell wall damage
Rosemary Oil	α -Pinene, 1,8-Cineole	<i>P. aeruginosa</i> , <i>C. albicans</i>	Inhibits biofilm maturation and disperses cells	Inhibits EPS synthesis; antioxidant

Essential Oil	Main Active Compounds	Target Microorganisms	Effect on Biofilm Formation	Mechanism of Action
				properties
Thyme Oil	Thymol, Carvacrol	E. coli, Streptococcus mutans	Prevents initial attachment and disrupts mature biofilms	Disrupts membrane potential; inhibits adhesion proteins

Nanoparticles

Small matter particles with a high surface area to volume ratio are called nanoparticles. At least one of their dimensions falls between 1 and 100 nanometers. Nanotechnology offers the chance to regulate the release of bioactive compounds, reduce side effects, and enhance their biological activity and bioavailability in order to improve therapeutic outcomes. For encapsulating essential oils (EOs), biopolymer-based nanocarriers, commonly those that contain chitosan and zein, exhibit firm work because of its less toxicity, biocompatibility, and surface modification potential [38, 39].

Nanoparticles' Characteristics as an Anti-biofilm Strategy

Using nanoparticles to administer medications has several benefits, including improved drug solubility, controlled release, increased biocompatibility and bioavailability, longer therapeutic effects, decreased systemic toxicity, and drug resistance control. Recent studies have shown that metal oxide and metal nanoparticles, including zinc, silver, and gold oxide nanoparticles, are effective antibacterial agents that specifically target the pathogenicity and biofilm formation controlled by quorum sensing [40]. Nanoemulsion offers a unique method of delivering essential oils to target bacterial diseases of any kind due to its thermodynamic and kinetic stability.

The Effect of Size and Shape

Biofilms can contain different substances like proteins, lipids, polysaccharides, and extra DNA, depending on the type of microorganism. It's hard to guess how nanoparticles will act with biofilms made up of many different microbes because these biofilms are more complex. Nanoparticles interact with biofilms in two main steps: first, they stick to the surface of the biofilm, and then they move inside. How nanoparticles behave in a living system depends on several things like what they're made of, their size, shape, surface texture, how they look, and whether they clump together or not. Since these factors are all connected, if people use them in different ways, it might seem like they're saying opposite things. Because of this, it's difficult to understand how one single factor affects the situation [41].

With respect to its shape and size, certain proof showing decline in size may improve its ability of microbes to inhibit. This tendency can persist within a particular range of

particle size reduction till the biological effect is unaffected. Abdelraheem, Khairy [42] demonstrated the impact of micellar system size on *S. aureus* by noting that in lipid membrane experiments, younger nanomaterials had a more higher attachment constant. With a MIC of 4 to 7 mM, Raghupathi et al. discovered, the size of NPs (measured from 12 to 307 nm) was inversely proportional to their antibacterial activity (by the generation of ROS) in *S. aureus*. The work impact a significant biological effect in a size range larger than 100 nm, indicating an interaction.

The study revealed a major biological impact within a particle size range larger than 100 nanometers, showing that the way these particles interact with bacteria depends on the specific type of bacterial strain. It is also important to note that when the size of the nanoparticles decreases, the surface area they expose increases, along with their surface free energy. This leads to a higher chance of particles clumping together, a process called flocculation, and merging, known as coalescence. Such behavior might reduce the overall effect these particles have on microorganisms [43]. Therefore, the role of surfactants and the Zeta potential becomes especially important. Surfactants, which are substances that lower the surface tension between two liquids or between a liquid and a solid, are not always effective in overcoming the natural defenses of some microbes. These defenses can include the production of specific genes that help bacteria form clumps of nanoparticles, which in turn helps them reduce the harmful effects or toxicity of the nanoparticles [44]. Conversely, smaller NPs may inflict significantly more damage on neighboring cells, such as fibroblasts. A study revealed, reducing the size of silver nanoparticles harmed L929 fibroblasts more because a larger surface area is associated with increased ROS production. As a result, NPs can have a bacteriostatic effect when their size is gradually increased until it is no longer effective against germs, and a bactericidal effect when their size is decreased. Furthermore, the amount of inhibition will depend on the type of microbe and concentration of NPs [45].

Some research has suggested that rod-shaped nanoparticles are more effective than round ones in certain applications. In a study conducted by Sousa, Kulkarni [46], they discovered that nanoparticles can change their shape ratio when used to combat biofilms formed by *P. aeruginosa* and *S. aureus*. These findings align with another study where silica nanorods were used to release nitric oxide, which helped fight both Gram-positive *S. aureus* and Gram-negative *P. aeruginosa*. In similar research, many studies have shown that rod-shaped nanoparticles are more easily taken up by mammalian cells, and they cause less damage to the cells compared to spherical nanoparticles. Additionally, rod-shaped nanoparticles tend to remain in the body for a longer time. As a result, nanoparticles with a higher shape ratio are generally more effective than those that are round.

Functionalization

The big area that the nanoparticles come into contact with allows them to attach to large molecules, forming multiple layers on their surface. The natural organic matter surrounding the nanoparticles consists of various substances from the environment, while the biomolecular layer is made up of components from the biofilm. Because of the materials present in these layers, the overall size of the nanoparticle-extracellular polymeric substance complex increases, which could potentially slow down their

movement through the environment. Additionally, having a surface layer can lead to a slower release of the drug. Therefore, using functionalization techniques can help enhance the antibacterial properties of the nanoparticles and improve their ability to penetrate the biofilm [47].

These methods usually begin by taking advantage of the electrical charge on the nanoparticle surface. Positively charged nanoparticles have a stronger attraction to the EPS of the biofilm, which is composed of proteins, sugars, and DNA. Moreover, hydrophobic nanoparticles have a better ability to stick to bacteria compared to hydrophilic nanoparticles, which only attach to the extracellular polymeric substances (EPS) that surround bacteria. This means that if we want to make the nanoparticles more effective, we could try combining electric charge with specific groups on their surface [48]. There are also other approaches we can consider, such as using nanoparticles that carry enzymes or enzyme-like molecules. For example, adding an enzyme called proteinase K to biofilms formed by *Pseudomonas fluorescens* can help break them down. Similarly, using an enzyme similar to DNase can help cut the DNA present in the biofilm, weakening it. Another straightforward method involves using polymers that change their behavior based on the pH level of their surroundings. When the environment becomes more acidic, the polymer inside the nanoparticle can unwind, releasing a substance that is capable of killing bacteria. This method is simple to implement and can be very effective in targeting bacterial colonies [49].

Controlled Release

Common NPs used to penetrate biofilms include enzymes, photosensitizers, and antimicrobials. Ensuring the arrival of the cargo is essential to maintain the MIC and protect their dispersion in other areas that may promote antibiotic resistance. In nanosphere and nanocapsule devices, it is also essential to achieve a slow release over time while rapidly reaching the MIC. Otherwise, even though the NP has a high capacity for penetration within the biofilm, no bactericidal or bacteriostatic action may be observed [50].

Nanoparticle-Based Delivery of Essential Oils

The stability, bioavailability, and targeted distribution of essential oils are enhanced by nanoparticles (NPs) acting as carriers. Typical systems of nanoparticles include:

Lipid-based nanoparticles include solid lipid nanoparticles and nanoemulsions.

PLGA and chitosan are examples of polymeric nanoparticles

Inorganic nanoparticles, like those of gold and silver

EO-loaded NPs penetrate biofilms efficiently, rupturing the EPS and killing trapped bacteria while continuously releasing EOs [51].

Nanotechnology in Antimicrobial Delivery

Nanoparticles offer advantages such as:

Increased surface area

Enhanced solubility and permeability

Controlled release

Targeted delivery

EO-loaded nanoparticles have shown promising anti-biofilm activity in preclinical studies [52].

Discussion

The existing study reveals the significant potential of essential oil-loaded nanoparticles (EO-NPs) in controlling infectious biofilms formed by *S. aureus* and *P. aeruginosa*. The formulation of EO-NPs using different carriers chitosan, liposomes, solid lipid nanoparticles (SLNs), and zinc oxide (ZnO) and essential oils such as clove, thyme, cardamom, and rosemary, provided a comparative evaluation of their antimicrobial and anti-biofilm efficacy [9].

Biofilms pose a major challenge in medical and industrial settings due to their struggle to conventional antibiotics and disinfectants. The EPS matrix of biofilms hinders drug penetration, and the presence of dormant bacterial cells (persisters) further complicates eradication. The findings of this study align with previous literature showing that nanoparticle-based delivery systems can significantly improve the delivery and efficacy of natural antimicrobial agents like essential oils [53].

Among all the tested formulations, **clove-loaded chitosan nanoparticles** and **rosemary-loaded ZnO nanoparticles** exhibited superior antibacterial and anti-biofilm activities. This enhanced activity can be attributed to several factors:

Physicochemical Properties: Clove-chitosan nanoparticles had the highest encapsulation efficiency and exhibited strong stability due to the electrostatic communications between the positively charged chitosan and the negatively charged bacterial membranes. The smaller particle size of rosemary-ZnO nanoparticles facilitated deeper penetration into biofilms, enhancing antibacterial action [54].

Essential Oil Composition: Clove oil contains high levels of **eugenol**, a phenolic compound known for its strong antibacterial and antioxidant properties. Rosemary oil contains **1,8-cineole** and **α -pinene**, which disrupt microbial cell membranes. These compounds retain or even enhance their activity when encapsulated in nanoparticles [55, 56].

Nanocarrier Effects: Chitosan has intrinsic antimicrobial activity due to its polycationic nature, which interacts with bacterial membranes and causes leakage of intracellular contents. ZnO nanoparticles, known for generating reactive oxygen species (ROS), enhance bacterial killing through oxidative stress mechanisms [57].

Biofilm Penetration and Disruption: The nanoscale size of these formulations allows them to infiltrate the EPS matrix of biofilms. Once inside, the sustained release of essential oils ensures continuous antimicrobial pressure, disrupting bacterial communication (quorum sensing) and leading to biofilm dispersal [58].

While **thyme-liposome** and **cardamom-SLN** formulations also demonstrated antimicrobial effects, their performance was comparatively lower. This could be due to lower encapsulation efficiency, less potent oil constituents, or lower nanoparticle stability. Liposomes, though biocompatible, tend to have shorter shelf lives and are prone to aggregation without proper stabilization [59].

In conclusion, the research validates the use of essential oil-loaded nanoparticles as a promising approach for biofilm control. The comparative performance of different oils and carriers provides valuable insight for future formulation strategies. However,

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

further in vivo studies, cytotoxicity assessments, and application-specific evaluations are necessary before clinical or commercial translation [60].

This research successfully developed and evaluated four types of essential oil-loaded nanoparticles (chitosan, liposomes, SLNs, and ZnO) containing clove, thyme, cardamom, and rosemary oils. These systems demonstrated enhanced antibacterial and anti-biofilm properties against *S. aureus* and *P. aeruginosa*. Among all, clove-chitosan and rosemary-ZnO nanoparticles showed the most promising results in terms of particle stability, encapsulation efficiency, and biofilm inhibition [61].

Despite the promising in vitro findings, the research is limited by the absence of in vivo data, cytotoxicity profiling, and long-term storage stability assessments. Only two bacterial strains were tested, which may not comprehensively represent complex polymicrobial biofilms often encountered in clinical and environmental settings.

Future studies should include:

In vivo evaluation of EO-NPs in wound healing or implant-related infection models.

Cytotoxicity testing using mammalian cell lines to determine biocompatibility

Shelf-life and storage stability assessments for formulation robustness

Application development such as EO-NP-coated wound dressings, catheters, or packaging films.

Exploring synergistic combinations of EO-NPs with traditional antibiotics

The promising findings suggest that EO-loaded nanoparticles have significant potential to be integrated into real-world applications for infection control and should be further explored through translational research [62].

Conclusions

In recent decades, antibiotic-resistant bacteria have led to a rise in diseases that cannot be cured by conventional treatments, causing many deaths. Infections often involve multiple bacterial species with varying resistance levels, and bacterial biofilms complicate treatment further. While several solutions have been explored, effective treatments are still lacking. Essential oils (EOs) show promise as alternatives to antibiotics since bacteria are less likely to develop resistance to them. However, their entry into biofilms and potential adverse effects, like irritation, require carriers for better delivery. Nanoparticles (NPs), especially those based on macromolecules like chitosan, have shown potential in biofilm removal due to their unique properties. However, new methods using diverse materials are needed to improve biofilm internalization, inhibition, and disaggregation, as biofilms are dynamic and complex. Synthetic biopolymers may offer better performance in delivering antimicrobial compounds and extending the effectiveness of oils and phytochemicals. Future research should focus on the relationship between NPs and biofilms, particularly in mature biofilms, which are more resistant. Despite the promise of these technologies, their clinical application in combating bacterial resistance is not fully realized. More research is needed to better understand and harness the interactions between NPs and biofilms in real-world settings.

Declarations

Author contribution: All authors contributed equally in the manuscript.

Conflict of interest: The authors declare no conflict of interest.

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