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A Comprehensive Review on Antimicrobial Efficacy of an Essential Oil 'Eugenol'

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Abstract

The increasing need for effective antimicrobial agents has become paramount due to the rising prevalence of antibiotic resistance among pathogens. In the current scenario, the overuse and misuse of antibiotics in clinical and agricultural settings have accelerated the development of resistant strains, creating an urgent demand for alternative therapies. Antibiosis, the mechanism by which antibiotics exert their effects, primarily involves inhibiting cell wall synthesis, disrupting protein production, or interfering with nucleic acid replication. While synthetic antibiotics have dominated the market, there is growing interest in natural sources of antibiotics, particularly plant-derived compounds. One such compound, eugenol, found in essential oils, has shown promising antimicrobial properties. Eugenol not only inhibits various foodborne pathogens but also enhances the efficacy of conventional antibiotics by lowering their minimum inhibitory concentration (MIC), making it a valuable candidate for use in combination therapies.

Keywords: Antibiosis; Antimicrobial resistance; Bioactivity; Essential oil; Eugenol.

INTRODUCTION

One of the main methods to improve beneficial biological effects and minimize potential adverse effects is to modify the molecular structures of naturally occurring physiologically active chemicals.[1] According to estimates from 1998, 60% of anticancer and anti-infective medications on the market or in clinical trials came from natural sources through structural alterations.[2] More recent data from December 2014 reveal that 138 (or roughly 58.30%) of the 237 anti-infectious agents (antibacterial, antifungal, parasitic, and antiviral) recognized by public health agencies globally, are natural products or products derived from natural products. Therefore, it is obvious that this is a field of study with excellent promise for discovering new medicines.[3] Eugenol was initially discovered in 1929 and commercial manufacturing of the natural chemical, which is utilized as a target

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molecule for the creation of bioactive compounds, started in the United States in 1940. Although it can be made synthetically, *Ocimum tenuiflorum, Cassia fistula, Zieria smithii,* and *Pimenta racemosa* are the primary sources from which it is harvested. Eugenol is light-yellow oil with a spicy flavor and a clove-like aroma, an allyl-phenol type phenyl propanoid. It has an extensive applications in the pharmaceutical, food, agricultural and cosmetics industries[4] and when tested against the fungi Cladosporium spp.[5] it shown potential antibacterial and antioxidant activities.[6] Other actions, such as antiviral, anti-inflammatory and inhibitor of platelet aggregation, have been documented in the literature. Additionally, it qualifies as a key provider of leishmanicidal due to its anti-Leishmania efficacy and minimal cytotoxicity.[7] Eugenol is a target molecule for structural changes to generate substances with medicinal properties because of its wide range of biological action.

Currently, bacterial infections and cellular oxidation stand out among the different clinical diseases, both of which have significant effects on public health. Commercial antibiotics are frequently unable to stop bacterial growth due to the resistance development and mutation mechanisms in microbes, leading to failures in the treatment of multi-resistant bacteria. Consequently, bacterial resistance has emerged as a global public health issue.[8] Aerobic organisms have the capacity to generate free radicals, which when in excess can start a cascade of reactions that harm or kill cells. Therefore, many illnesses develop, including cardiovascular and neurological conditions. The application of antioxidants in diet has proven to exhibit a substantial impact on the prevention of various diseases in the battle against the damage caused by the generation of free radicals.[7] According to reports in the literature, eugenol reduces oxidative stress and has positive benefits on health.

Eugenol has diverse biological activities, the current review focuses mainly on the antimicrobial effects.

Antimicrobial activity

There are numerous natural, semi-synthetic, and artificial substances that have the potential to significantly alter metabolic and physiological processes. These substances include lactams and glycopeptides that alter the synthesis of cell walls, tetracyclines and macrolides that inhibit protein synthesis, sulphonamides that block the functions of certain metabolic pathways, and fluoroquinolones that disrupt DNA replication and translation.[9-10] Healthcare organizations are significantly concerned about bacterial resistance. The likelihood that bacteria will develop more complex resistance to antibiotics increases with increased global use of antibiotics. Because of this, some new modified strains seem to have decreased the likelihood that the treatments will be properly effective in patients, having serious repercussions that can lead to morbidity and mortality or clinical complications.[11] The ineffectiveness of the usage of antibiotics in healthcare is hampered by bacterial resistance, and there is strong evidence that improper use of antibiotics will ultimately lead to the development of resistance.[11] Additionally, the risk of managing immunity-compromised health conditions like cancer, HIV infection, surgery, and diabetes is reduced due to the lack of adequate access to effective antibiotics.[12] Although this is considered a natural process, there is a lack of information regarding the crisis of antibiotic resistance, accelerated by passive human activities such as inappropriate prescription practices, misuse of antibiotics by both physicians and patients, and incorrect diagnoses has significantly increased the rate of antimicrobial resistance.[13-14]

Mechanisms for antimicrobial activity

Understanding the modes of action of antimicrobial medications is crucial to understanding the processes of resistance. Antimicrobial agents concentrate on particular significant bacterial functions. Different antimicrobial agent classes kill or inhibit bacteria in different ways. It can categorize the different ways that antimicrobial agents function into the following groups.

Preventing the cell wall from being produced

It is essential for preserving the shape of the bacterial cell and protecting the bacteria from lysis caused by the high intracellular osmotic pressure is crucial, due to the elastic macromolecule that constitutes the bacterial cell wall.[15,16] Peptidoglycan is the primary component of the bacterial cell wall, consists of long glycan chains made of N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), which are cross-linked by short peptides (composed of four amino acids). This cross-linking is facilitated by enzymes known as transpeptidase and carboxypeptidase, collectively referred to as "Penicillin-Binding Proteins." Many antibiotics, including -lactam and glycopeptides, focus primarily on this physical

barrier.[16] Antibacterial drugs include the lactams (penicillin's, cephalosporins, carbapenems, and monobactams) and the glycopeptides (vancomycin and teicoplanin), which prevent the growth of bacterial cell walls.[17]

Preventing the synthesis of protein

Antibiotics that stop protein synthesis are essential for bacterial growth and development. When protein synthesis is suppressed, cell proliferation is either halted or slowed down.[18]

Tetracyclines, macrolides, and aminoglycosides were preventing the synthesis of new proteins by attaching to the 30S subunit and some of the antimicrobials can cause an error in protein synthesis.[19]

Preventing the synthesis of genomic material

Broad-spectrum antibiotics known as fluoroquinolones are used to treat gram-positive, gram-negative, and anaerobic bacteria. In gram-negative bacteria, fluoroquinolones act as inhibitors of the DNA gyrase enzyme, which is essential for the start of DNA replication, and in gram-positive bacteria, as an inhibitor for the topoisomerase IV enzyme, which is essential for daughter-cell segregation (decatenation).[20-21] Quinolone antibiotics work to inhibit DNA supercoiling by interacting with either topoisomerase IV or II. which results in double-stranded DNA breaking down and cell death. Either using a method that depends on protein synthesis or one that doesn't.[22]

Inhibition of bacterial enzymes or metabolic pathways

The folate biosynthetic pathway is an excellent target for antibiotics because eukaryotic cells acquire folate through an active transport system, whereas microorganisms must synthesize it de novo. To synthesize folate, the enzyme dihydropteroate synthase (DHPS) requires para-aminobenzoic acid (PABA). Sulphonamides have been observed to prevent the application of para-aminobenzoic acid (PABA) in bacterial folate synthesis.[23] Sulphonamides share structural similarities with PABA and act as a substitute substrate for PABA as a competing inhibitor to stop the growth of bacteria by consuming the folate pool.[13] The final enzyme in the sequence, dihydrofolate reductase (DHFR), has served as a target for dihydrofolate pyrimidine antibiotics, like trimethoprim.[24-25]

Alteration to the bacterial membrane's structure

The Polymyxin molecules bind to the cell membrane of bacterial peptidoglycan and change the structure of the membrane to make it more permeable. These modifications cause an osmotic imbalance that results in the expulsion of cellular compounds, the inhibition of respiration, and quick water absorption that results in cell death.

Antimicrobial Resistance (AMR)

Historically, it is well established that, humans have fought a protracted battle with microorganisms, particularly bacteria, which has resulted in substantial morbidity and mortality in various human populations all over the world. Penicillin was a powerful antimicrobial agent for bacteria in the early 1940s. Consequently, it was widely used by people to combat numerous infectious diseases. Penicillin's efficacy has decreased due to overuse, though, as bacteria have begun to develop a number of resistance mechanisms.[18] The capacity of microorganisms to persist and be viable in the existence of antimicrobial agents is known as antimicrobial resistance (AMR). AMR has been developed within the bacteria as a result of prolonged and intensive use of antibiotics over time. The natural genetic evolution of bacteria to resist antibiotics has reached paradoxical heights in the twenty-first century, and this has made AMR a serious health threat with potential global repercussions that calls for immediate action. There are multiple ways in which they develop resistance are by intrinsic/acquired resistance, acquired itself contains alternative methods to develop resistivity through enzyme modification or inactivation of microbicidal molecules inside the cells and also by altering the target site for binding can further impact their efficacy.[9]

Kinds of antimicrobial resistance

Intrinsic Resistance: Some particular bacterial genera (or species) have distinctive structural/ functional traits that give them antibiotic resistance. These bacterial populations often lack a target site for the specific antibiotic, making it ineffective. For instance, Mycoplasma species are resistant to glycopeptides and lactam antibiotics because they lack a cell wall. Additionally, the presence of an outer membrane hinders the entry of antibiotics into bacterial cells. Further, this resistance may be due to the presence of an export system (such as the AcrAB-TolC system) or the ability of certain bacterial species to produce enzymes that inactivate antibiotics, such as the AmpC β-lactamase in E. coli.[26] Acquired Resistance: In this situation, naturally susceptible bacteria can become resistant to some antibiotics by acquiring their

genetic material from other bacterial strains. The three primary mechanisms of acquired resistance include the following.[26]

Altering enzymes or rendering antimicrobial agents inactive

Both gram-positive and gram-negative bacteria are capable of modifying enzymes or render antibiotics ineffective. In enzymatic modification, acetyl, adenyl, or phosphate groups are added to specific sites on an antibiotic by bacterial enzymes. This chemical alteration inactivates the antimicrobial agent, preventing it from binding to its target site.[27] For instance, phosphorylation happens in macrolides, whereas aminoglycosides either undergo acetylation, adenylation, or phosphorylation. In enzymatic inactivation, antibiotics are primarily degraded through the hydrolytic cleavage action of bacterial enzymes. (such as lactamases against penicillin and cephalosporins) decreased intracellular antimicrobial agent accumulation. To reduce the buildup of antibacterials within bacterial cells, bacteria either use reduced influx or enhanced efflux. Antibiotics, such as tetracyclines and lactam, which enter E. coli by the OmpF and carbapenems, which enter *Pseudomonas aeruginosa* by the OmpD, are thought to enter bacteria through porins, an outer membrane protein (OMP). Porin genes may be downregulated, structurally altered, or even functionally deleted 26. The resistance-nodulation-cell division (RND) family of transporters, such as the AcrAB-TolC system in *E.coli*, is a type of increased efflux mechanism found primarily in Gram-negative bacteria. These transporters actively expel antibiotics from the cells and are commonly used by many bacterial species to remove toxic substances produced during cellular metabolism.[26-28]

Alterations at the antimicrobial agents target sites

Among the changes at the target sites are: Fluoroquinolone resistance is primarily caused by mutations in the quinolone-resistance-determining region (QRDR) in the DNA gyrase (topoisomerase II and topoisomerase IV). This can happen in both gram-positive and gram-negative bacteria. For example, Erm methylases, which target macrolides, lincosamides, and streptogramin B antibiotics, are known to be highly effective in developing resistance in both Gram-positive and Gram-negative bacteria. Additionally, the methylation of the cfr gene has been used to detect resistance in a variety of bacteria, including *Proteus vulgaris, Staphylococcus spp., Enterococcus spp., Bacillus spp., and E. coli.*[29] Resistance to sulphonamide and trimethoprim is frequently caused by the replacement of an origin target that is susceptible to a particular antibiotic with a drug-resistant target. Sul1, Sul2, and Sul3 are genes for dihydropteroate synthases in gram-negative bacteria, which are resistant to sulphonamides.[34,45] Additionally, the mecA and mecC genes in Staphylococcus spp. produce an alternative penicillin-binding protein with a significantly reduced affinity for all β-lactam antibiotics. This alteration disrupts the elongation process and prevents protein synthesis 30. The process of dissemination of resistance gene by Transformation, Transduction, Conjugation, resulting in formation of MDR and XDR multiple drug and extensive drug resistance bacterial strain respectively. The former is resistive to at least one or two drug of any class whereas later is susceptible to one or few class of drug only.

Antimicrobial Activity of Eugenol

The free OH group in the structure of eugenol has been linked to its possible antibacterial effects against a variety of species, including *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Escherichia coli*. Eugenol is a hydrophobic molecule that can easily pierce the lipopolysaccharide cell membrane and enter the cytoplasm. It is thought that this property of eugenol causes it to work against Gram-negative bacteria by disrupting the cytoplasmic membrane. Once within the cell, it has the potential to modify the cell's structure and cause intracellular components to seep out. It has been proposed that the hydroxyl group in eugenol inhibits protease, histidine decarboxylase, and amylase activity in Enterobacter aerogenes by binding to these enzymes.[31] Similarly, eugenol has been found to potentially inhibit the activity of membrane bound ATPase in Listeria monocytogenes and Escherichia coli. Furthermore, it has been observed that traditional antimicrobials exhibit enhanced effectiveness when used in combination.[32] Additionally, eugenol is believed to generate intracellular reactive oxygen species (ROS), which can damage cells by inhibiting cell growth, rupturing cell membranes, and causing DNA destruction.[33] *Streptococcus agalactiae* (planktonic GBS—group B streptococci strain) human isolates, including those resistant to erythromycin and clindamycin, were observed to develop less rapidly when exposed to eugenol.[34] The GBS planktonic cells exhibited leakage of proteins and lipids from the cytoplasm and rupture of the cell membrane after a five-hour incubation with 0.125% to 0.5% eugenol.[35] The minimum inhibitory concentration (MIC) for the derivatives

was found to be 500 µg/mL, compared to 1000 µg/mL for eugenol. Additionally, eugenol derivatives demonstrated stronger antibacterial activity than eugenol itself. The derivatives were synthesized by adding functional groups to the double bond of the allyl group or by esterifying the hydroxyl group (-OH) with various carboxylic acid derivatives.

Eugenol has demonstrated antiviral activity alongside its potent antibacterial properties. It works synergistically with acyclovir to inhibit the herpes virus in vitro, including HSV-1 and HSV-2 (Herpes simplex virus types 1 and 2), by limiting viral infection and preventing replication. Eugenol has also been found to possess antifungal activity against a range of fungal strains in vitro, including *Candida albicans, Aspergillus niger, Penicillum glabrum, Penicillum italicum, Fusaria oxysporum, Saccharomyces cerevisiae, Trichophyton mentagrophytes, Lenzites betulina, Laetiporus sulphurous, and Trichophyton rubrum*. Eugenol is hypothesized to interfere with the function of cell membranes in fungi, suppress virulence factors, and impede the development of fungal biofilms.[36] Sharifzadeh and Shokri used the broth microdilution test and the checkerboard microdilution method to assess the antifungal activity of eugenol and its potential synergistic effects with voriconazole against Candida strains isolated from mares' reproductive systems. The minimum inhibitory concentrations (MICs) of eugenol were 400–800 µg/mL for Candida tropicalis and 200–400 µg/mL for Candida krusei. When combined with voriconazole, eugenol showed synergistic effects against *Candida tropicalis (83.3%)* and *Candida krusei (77.7%)*, with no evidence of antagonistic activity. As a result, eugenol has the capability to be an effective antifungal medication for treating genital Candida yeast. Additionally, the combination of eugenol and voriconazole could be effective in reducing antimicrobial resistance in mares with genital candidiasis.[32] According to a study on eugenol, when paired with different antibiotics like vancomycin, penicillin, ampicillin, and erythromycin, the MIC values could be reduced by as much as 5-1000 times more compared to when the individual antibiotics were used alone. Furthermore, it has been shown that eugenol enhances the bacterial cell membrane-damaging effects of lysozyme and SDS. Limiting the usage of antibiotics to natural substances like eugenol appears to be a feasible answer because the widespread use of antibiotics contributes to the emergence of antibiotic-resistant bacterial strains.[37] Eugenol's capacity, as an ingredient in essential oils, to remove bacterial biofilm has been investigated, along with that of other substances such trans-cinnamaldehyde, citronellol, and terpineol. A three-dimensional microcolony of bacteria that is embedded by an extracellular matrix is known as a bacterial biofilm.[38] A serious issue is bacterial biofilms. For instance, they can proliferate on the surface of food products in the food industry, contaminating the food and potentially causing disease. Effective solutions are therefore being sought to resolve this issue.[39] The aforementioned substances, which are elements of essential oils, were tested by Olszewska *et al.*[39] for their capacity to prevent the development of the *Escherichia coli* biofilm. The study utilized platelet count, resazurin test results, and Syto® 9/PI (propidium iodide) staining, along with flow cytometry (FCM) and confocal laser scanning microscopy (CLSM). With a concentration of 3 mM, eugenol significantly reduced the metabolic behavior of bacterial cells that are a part of the biofilm (49%), as well as their capturability (84%). However, eugenol demonstrated the least capacity to harm the microbial cell membrane when compared to the other compounds tested. Another frequent source of bacterial infection is the biofilm that bacteria create on biomaterials and medical implants. A hydrophilic copolymer system based on eugenol was discovered to successfully stop the growth of these bacteria in studies. Eugenol affects bacterial biofilms in general by inhibiting biofilm development and decreasing the vitality of cells that produce biofilms. The inactivation of bacterial biofilm cells, cell dispersion within the biofilm matrix, and reduction of biofilm-associated gene expression, such as that of the pgaA gene, were additional impacts. Eugenol may also limit the development of biofilms and impede the synthesis of bacterial virulence factors such violacein, elastase, and pyocyanin. Additionally, *Salmonella enteritidis* and other multi-resistant bacteria seem to be susceptible to its effects.[40] Antimicrobial activity against carbapenem-resistant *Klebsiella pneumoniae* (CRKP) was also demonstrated by Qian *et al*. As a result of its resistance to antibiotics and antimicrobials, *Klebsiella pneumoniae* is a highly pathogenic bacterium that poses a serious threat to both humans and animals. This bacterium demonstrates a variety of virulence traits, including as the capacity to form biofilms and the presence of outer membrane proteins and capsular polysaccharide. Finding a powerful drug to fight or weaken this virus is crucial since carbapenem-resistant CRKP strains pose a particularly serious threat. Given that it has shown multidirectional activity, eugenol appears to be a promising

chemical with antibacterial activity against this pathogen. By using the agar dilution method, the minimal inhibitory concentration (MIC) of eugenol was found to be 0.2 mg/mL against the four tested CRKP isolates. The extent of cell damage and the number of damaged cells both increased with higher concentrations of eugenol, which is significant. Eugenol's antibacterial effect was caused by damage to the cell membrane, this damage included membrane rupture and cell enlargement, hyperpolarization of the membrane and increased membrane permeability, and, eventually, leaking of intracellular components from CRKP cells. Eugenol has been employed in dental and oral care since ancient times. It has disinfectant properties, antimicrobial activity against bacteria linked to dental caries and periodontal disease, and has been found to relieve local pain from conditions like pulpitis and dentinal hypersensitivity as a topical analgesic. It is mixed with zinc oxide in dentistry to create an amorphous chelate substance that is used to cover the pulp inadvertently, dress endodontic therapy, and temporarily fill cavities. Furthermore, it can be used in liquid form to fill root canals with specialty pastes such mummification pastes (such as Caryosan and Endomethazone). Additionally, eugenol is sometimes applied to the gums to numb them prior to the insertion of dentures. Eugenol is frequently used in pharmaceuticals, food, cosmetics, and as a local antiseptic and analgesic due to its multidirectional action and reputation as a usually safe chemical at low concentrations. In addition, it is frequently found in home products including soap, perfume, skin care items, and cigarettes where it serves as a flavor and scent. In addition to being a pesticide and fumigant, it is also employed as a preservative to safeguard foods against microbes. The maximum daily intake of eugenol or clove oil for humans is 2.5 mg/kg body weight, according to the Joint Food and Agriculture Organization/WHO Expert Committee on Food Additives.[41] Due to its beneficial effects on health, eugenol is also used to treat joint pain and infections of the upper respiratory and gastrointestinal tracts. Additionally, eugenol is a component of several medications used to treat upper respiratory tract mucosal inflammation and for cold prophylaxis. Amol, Aromatol, or Olbas are a few examples of drugs that are frequently given as inhalation and aerosol therapy.[42] Eugenol has uses in food production and agriculture due of its multifaceted antibacterial and antifungal properties. An essential benefit is that its favorable effect is linked to low concentrations of active ingredient. Additionally, eugenol is effective against a range of foodborne pathogens (such as *Aspergillus ochraceus* and *Salmonella typhi*), therefore using it helps avoid acute food poisoning. Eugenol has shown to have anti-salmonella activity by reducing the permeability of the pathogen's cell membrane, which is followed by ion leakage, loss of cellular content, and eventually cell death.[32-41] Eugenol is therefore utilized as a biocontrol agent for grains in agriculture because it has been discovered that it may lessen Salmonella contamination of organic goods by inhibiting its proliferation in soil. Eugenol has the ability to prevent Aspergillus ochraceous from producing ochratoxin A. Fruits including strawberries, apples, and peaches as well as their juices are shielded from microbes' damaging effects by its antifungal characteristics *Staphylococcus aureus* is one of the primary prevalent infections associated with food. According to studies, eugenol inhibits the synthesis of toxic shock syndrome toxin 1, staphylococcal enterotoxin A and B, and alpha-hemolysin as well as the expression of these toxins in S.*aureus*.

However, eugenol can be pro-oxidative and potentially hazardous at high concentrations; the FAO deems levels below 2.5 mg/kg body weight as safe. Additionally, eugenol may occasionally cause allergic reactions, such as allergic contact dermatitis, particularly among dental professionals. Eugenol derivatives represent a significant area of research and hold promise as components in insecticides and pesticides.[41]

CONCLUSION

Eugenol is valued for its broad spectrum of biological activities and has various applications. It is commonly used as a fragrance in soaps and perfumes, but it also plays a role in pharmacology and medicine. Its primary uses include serving as a local analgesic, antiseptic, and anti-inflammatory agent in inhalation and aerosol therapies. Notably, eugenol has demonstrated therapeutic potential in medications for cancer treatment. Furthermore, it enhances the efficacy of several antibiotics, including vancomycin, penicillin, and erythromycin, by increasing their potency and lowering their minimum inhibitory concentration (MIC), which helps reduce pathogen resistance to antibiotics. Despite its benefits, careful dosing is required due to potential toxicity at high concentrations, and it may cause allergic reactions in sensitive individuals.

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