

Biological Activity of Eugenol and its Latest Progress in Antibacterial Application

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Abstract

In recent years, bio-based materials have been developed rapidly due to their wide range of sources and strong reproducibility. Eugenol, as a natural phenolic aromatic compound, has attracted much attention because of its many superior activities, and it has been used in various fields for a long time. In this paper, a series of biological activities of eugenol were reviewed, and the research progress of eugenol in the field of antibacterial was emphatically introduced, and on this basis, a summary and prospect were made.

Keywords

Eugenol; Bioactivity; Antibacterial Properties; Application Prospect.

1. Introduction

With the decrease in petrochemical resources, the preparation of bio-based polymer materials from renewable resources has gradually become a research hotspot. The raw materials of bio-based polymers are widely available and reproducible, and they have attracted much attention because of their broad application prospects. Among the natural renewable resources, the essential oils contained in plants are the sources of terpenes, terpenoids, or free monoaromatic compounds, and their chemical structures are various. Some[1]of these compounds are not only easy to be designed and utilize but also have a wide range of sources, low prices, and high biological activity, which can bring many conveniences to human life.

Eugenol is a natural product, which is mainly extracted from plants rich in clove oil, and can also be obtained[2, 3]by enzymatic hydrolysis of lignin. Because of its known antibacterial, antifungal, anticancer, anti-inflammatory, and antioxidant activities, it has long been used in the fields[4]of cosmetology, medicine, and pharmacology. It is worth mentioning that eugenol and its derivatives have become a hot research topic because of their superior antibacterial properties. Antibacterial materials also have a certain history of development in China, as early as the Shang Dynasty, people have known how to use mercury to treat epilepsy and other skin diseases. In the Warring States Period, the Book of Mountains and Seas had already recorded that "the disease of cattle and horses can be smeared by flowing grass, and the disease of cattle and horses can be smeared by fumigating grass", which means that sulfur and fumigating grass can prevent livestock and people from the m plague. Zhang Zhongjing's Treatise on Febrile and Miscellaneous Diseases in the Han Dynasty also recorded the method of using natural plants to sterilize and prevent infection. At present, antibacterial materials have been widely used in medicine, food, sewage, treatment, and other fields[5]. Materials with

excellent antibacterial properties of eugenol are widely used in food fresh-keeping packaging, anti-fouling and antibacterial coatings, oral rest, oration, and other fields. Therefore, in this paper, the biological activity of eugenol was summarized, the main application of eugenol in the field of antibacterial was introduced, and the future application of eugenol was summarized and prospected.

2. Biological Activity of Eugenol

2.1 Structure Composition and Characteristics

Eugenol, also known as eugenol, scientific name: 4-propenyl-2-methoxyphenol, English name: Eugenol, its chemical formula is $C_{10}H_{12}O_2$, colorless or pale yellow liquid, with strong clove aroma, slightly soluble in water, soluble in most organic solvents. Its chemical properties are very active. The eugenol molecule contains an unsaturated olefinic bond and a phenolic hydroxyl group, which is easily oxidized and can also undergo polymerization. When eugenol is heated in potassium hydroxide, the double bond of the propenyl group can be rearranged to obtain Isoeugenol, and after oxidation, the α -propenyl group can be cleaved to obtain Vanillin, which is the main component of flavoring agents and spices.

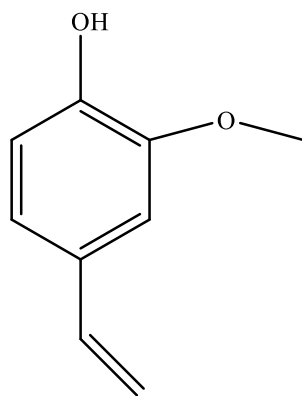


Figure 1. The structural formula of eugenol

2.2 Antioxidant Activity

Oxygen free radicals are ubiquitous in organisms, which are the products of metabolism. At present, academic circles initially believe that free radicals are important factors causing many diseases of aging organisms. Eugenol has good antioxidant activity, which can scavenge free radicals, inhibit the formation of active oxygen, and prevent the formation of active nitrogen. It can inhibit the formation of lipid peroxidation, increase the antioxidant capacity of cells, protect the internal structure of cells, and repair cells damaged by oxidation.

Li Jianfei[7] studied the antioxidation of eugenol. The results showed that eugenol had a strong ability to scavenge DPPH and ABTS free radicals and reduce iron. It also had a strong inhibitory effect on oil oxidation and a protective effect on DNA oxidative damage. Zhuang Junhui[8] determined the reducing power, free radical scavenging ability, and superoxide anion radical scavenging ability of eugenol. The results showed the IC_{50} dose of eugenol to hydroxyl radical was $152.52 \mu\text{g/ml}$. The IC_{50} for O_2^- was $215.99 \mu\text{g/ml}$.

The antioxidant activity of eugenol is closely related to its concentration. It has antioxidant activity at low doses, but it may promote oxidation at high concentrations, leading to the formation of free radicals. Therefore, it is necessary to control the concentration of eugenol when using its antioxidant activity under certain conditions. Combined with the actual application, it is possible to compound and modify eugenol to achieve the best antioxidant effect.

2.3 Anticancer Activity

It has been found that eugenol induces apoptosis of human promyelocytic leukemia cells (HL-60) by inducing mitochondrial permeability transition and reducing the level of anti-apoptotic proteins

through ROS generation and transduction of apoptotic signals. There is evidence that eugenol can be used as an antioxidant to affect cancer cells and prevent mutation and as a pro-oxidant to induce cancer cell death through a variety of signaling pathways. This substance, which has dual antioxidant and pro-oxidant effects, has a certain role[9]in the prevention of cancer formation and the treatment of cancer.

Moustafa[10]et al. Studied the application potential of eugenol in human cervical cancer cells, evaluated the synergistic potential of eugenol with chemotherapy and radiotherapy, and analyzed the caspase-3 activity by taking the cell survival rate after eugenol combined with cisplatin and X-ray as an example. The expression of various genes and proteins was examined by RT-PCR and Western blot analysis, and it was finally shown that eugenol showed synergy with cisplatin and X-ray.

Li[11]et al. studied the tumor inhibitory effect and potential mechanism of eugenol in human lung cancer cells and proved that eugenol inhibited the proliferation, migration, and invasion of lung cancer cells in vitro partly through PI3K/Akt pathway and MMP activity, indicating that eugenol could inhibit the cell viability of lung cancer cells. Can be used as a potential chemotherapeutic agent against human lung cancer.

The eugenol with the double effect can be further developed to further exert the anticancer effect of the eugenol by combining the eugenol with an anticancer drug or a cancer treatment process.

2.4 Analgesic and Anti-inflammatory Activity

The analgesic and anti-inflammatory activity of eugenol is mainly used in dental practice. When inflammatory mediators stimulate gingival nerves, they can cause severe periodontal pain, and eugenol can relieve dental pain, which is widely used as a local analgesic in dentistry, and has a certain anti-inflammatory effect[12]on periodontal tissue structure. Eugenol shares several pharmacological effects with local anesthetics, including inhibition of voltage-gated sodium channels (VGSC) and activation of transient receptor potential vanilloid subtype 1 (TRPV1), and its inhibitory effect on VGSC in trigeminal ganglion (TG) neurons were found in a study[4]based on a rat tooth model.

Ahmad[13]et al. developed eugenol nanoemulsion-gel (EUG-NE-Gel) with rats as the research object. Compared with traditional preparations, nanoformulations have many advantages, such as enhancing drug solubility and permeability to periodontal mucosa, reducing drug dosage, and reducing side effects. EUG-NE-Gel can improve tooth mobility, and gingival index, and significantly reduce inflammatory infiltration, which is related to the anti-inflammatory effect of eugenol.

In addition to its wide use in dental clinics, eugenol can also be used as a local anesthetic for other pathological pain, can replace some non-steroidal anti-inflammatory drugs to treat some diseases[4], and can also be used to synthesize new selective drugs to better combat inflammation.

2.5 Antimicrobial Activity

At present, many experiments have proved that eugenol has antibacterial activity on many species. It has broad-spectrum antibacterial activity against Gram-positive bacteria (such as *Listeria monocytogenes*, *Staphylococcus aureus*, etc.) And Gram-negative bacteria (such as *Escherichia coli*, *Vibrio vulnificus*, etc.).

Liu et al.[14]studied the effect of eugenol on *Listeria monocytogenes*, and found that eugenol can reduce the formation of biofilm by inhibiting the quorum sensing system. It can down-regulate the quorum sensing genes (*agrA*, *agrC*, and *agrD*) at a quarter of the minimum inhibitory concentration (MIC), and inhibit the expression of related genes. Thus affecting the growth of bacteria.

Li Haixian[15]studied the bacteriostatic mechanism of eugenol on *Staphylococcus aureus* and found that eugenol could increase the permeability of cocci, change the morphology of cocci, significantly inhibit the activity of MDH and SDH enzymes of *Staphylococcus aureus*, and affect the growth of *Staphylococcus aureus* through multiple targets.

Jeyakuma[16]et al. studied the antibacterial mechanism of eugenol against E. coli and found that the MIC range of eugenol was 0.0312 ~ 8 µg/ml, which could effectively reduce E. coli to an undetectable level within 4 hours. Moreover, eugenol can destroy the cell membrane of bacteria, change the permeability of the cell membrane, and lead to the leakage of intracellular substances.

Luo[17]et al. studied the antibacterial and biofilm removal ability of eugenol against *Vibrio vulnificus* and found that eugenol had a good antibacterial effect against *Vibrio vulnificus*, and the minimum inhibitory concentration was 0.2 mg/mL. The bactericidal mechanism was the accumulation of reactive oxygen species (ROS) and the increase of malondialdehyde (MDA) content. At the same time, eugenol can reduce the number of biofilm of *Vibrio vulnificus*, the number of polysaccharides, and living cells in the biofilm, indicating that eugenol has a significant biofilm removal effect on biofilm.

Not only that, eugenol is a potential antifungal agent. Miri[18]et al. Studied the effects of eugenol and menthol on the growth and spore germination of *Aspergillus parasitics*, and the results showed that both of them could reduce the germination rate of fungal spores at a certain concentration, indicating that eugenol could reduce fungal infection.

Because of its excellent antibacterial effect and its wide range of sources, eugenol occupies a place in the natural compound antibacterial, people's demand for antibacterial materials is gradually expanding, so the application field of eugenol antibacterial activity is also gradually expanding, and it can be found in food, medicine, and other production and life fields.

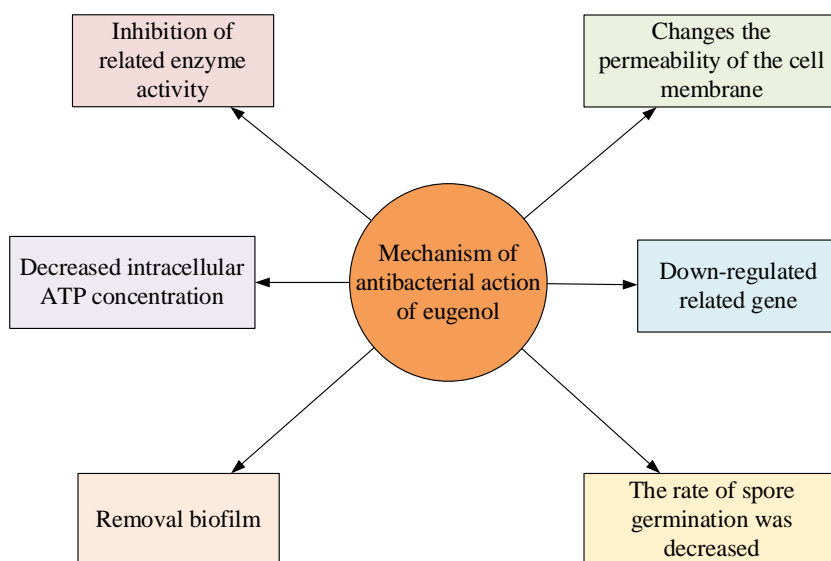


Figure 2. Antibacterial mechanism of eugenol

3. Derivatives of Eugenol

Eugenol is a compound that has attracted much attention from researchers. It has a general structure of compounds and has a wide range of applications. Because eugenol has many advantages, we can use its different activities to design and synthesize different derivatives, make full use of the diversity of eugenol in structure, chemistry, and biology, and its superior natural activity will be used by everyone.

Zamli[19]et al. studied the preparation of 9 eugenol derivatives from eugenol by different methods, named K1 ~ K9, in which K1 was prepared by inert treatment of eugenol with potassium carbonate and 4-isopropyl benzyl chloride, and K2 ~ K9 were prepared from trimethylamine, acyl chloride, and

other raw materials, and purified by column chromatography. A series of derivatives synthesized by the method can be considered potential medicaments for resisting acanthamoeba infection.

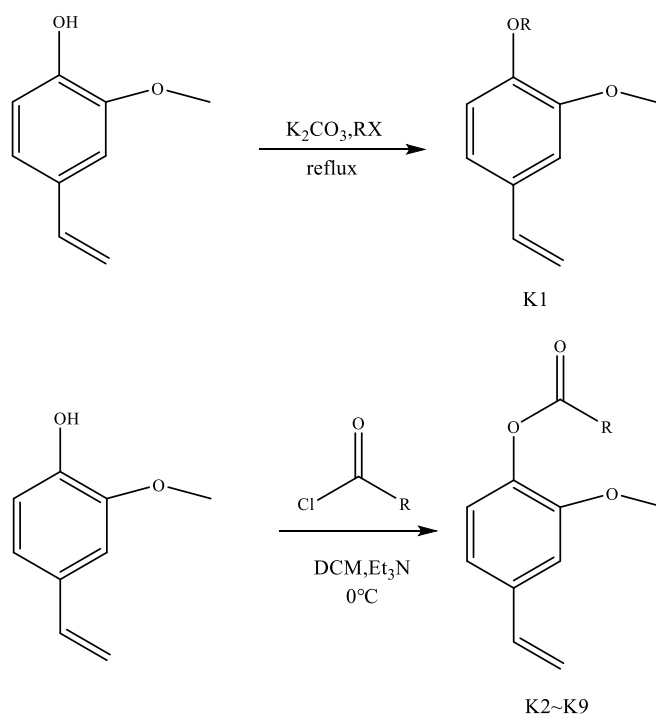


Figure 3. Synthesis of K1 ~ K9 eugenol derivatives

In addition, eugenol can also be used to prepare bio-based thermosetting resins to replace traditional thermosetting resins, reduce the dependence of traditional polymer materials on petrochemical resources, and achieve the dual effects of saving resources and protecting the environment.

Based on eugenol, two eugenol-based monomers EM2G, and EM3G were designed and synthesized by Dai Jinyue[20], which were used as reactive diluents to copolymerize with epoxidized soybean oil acrylate (AESO) to prepare a series of vegetable oil-based thermosetting resins. After testing, it was found that the higher the content of EM2G and EM3G, the better the performance of the resin.

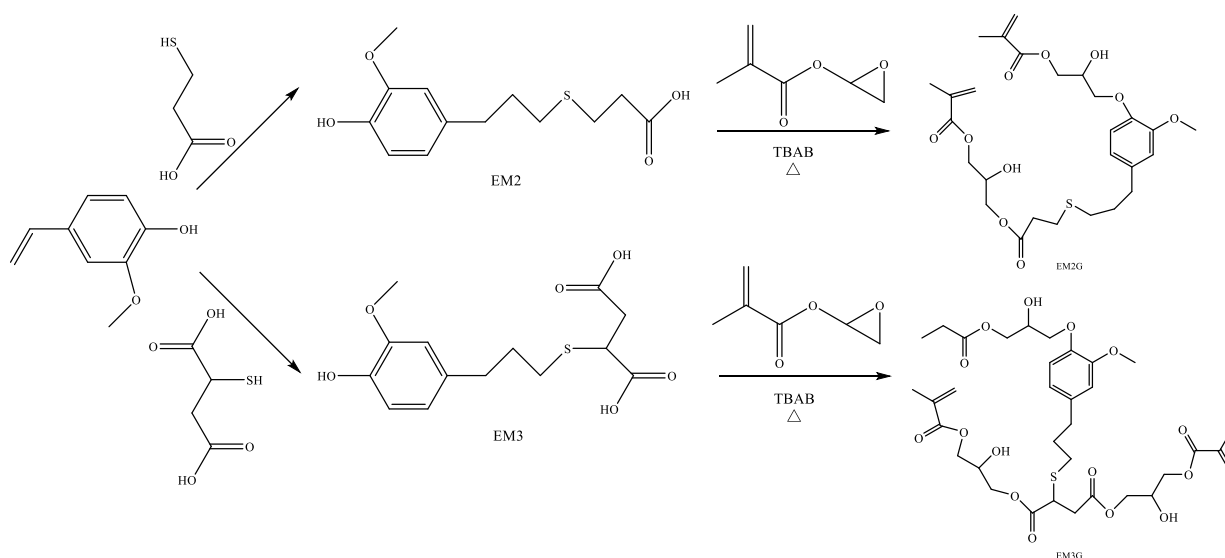


Figure 4. Synthetic route of EM2G and EM3G

Jin Shenglin designed a new bio-based epoxy resin monomer[21]with eugenol as raw material. The eugenol biphenyl intermediate (DEU) with 100% bio-based content was obtained by the oxidative coupling of eugenol, and the eugenol-based biphenyl epoxy monomer (DEU-EP) was finally obtained by the glycidyl reaction of the intermediate with epichlorohydrin. The cured monomer was tested, and it was found that the cured sample had high storage modulus and surface hydrophobicity.

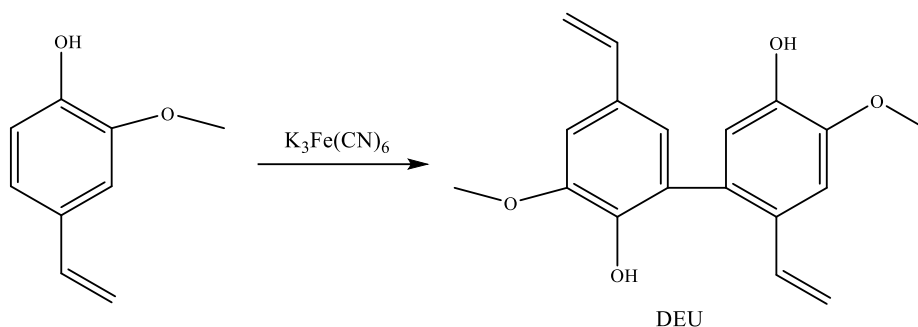


Figure 5. Synthesis of DEU intermediate

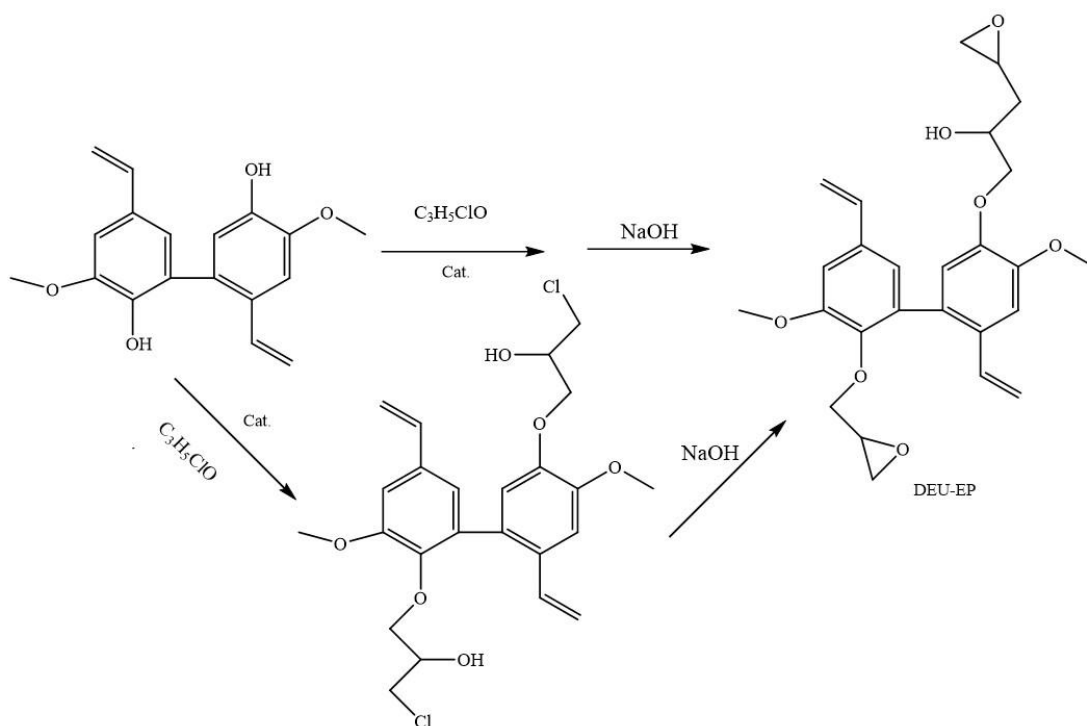


Figure 6. Synthesis of DEU-EP

4. Application of Eugenol in Antibacterial Field

4.1 Food Fresh-keeping Packaging Field

In recent years, people's demand for food safety is getting higher and higher. Inevitably, food packaging is easily invaded by bacteria and other microorganisms. Food spoilage caused by microbial invasion not only reduces the nutritional and hygienic quality of food, but also may endanger human health, and cause huge waste of resources and environmental pollution. Some natural compounds extracted from plants, animals, and microorganisms can be used as natural antibacterial[22]agents with low harm to the human body. Cui[23]et al studied the inhibitory mechanism of eugenol on *Bacillus cereus* and its spores, and expanded the application scope of eugenol, a naturally active substance, in the pollution control of food-borne pathogens. Laid a theoretical foundation. At present, this natural antibacterial material has become an important part of antibacterial packaging.

Lin[24] et al. Prepared eugenol-loaded sodium caseinate and trimethyl chitosan composite nanoparticles (ESTNPs) and gelatin films loaded with ESTNPs, both of which have good compatibility. The incorporation of EST NPs significantly improved the light, water, and oxygen barrier properties and thermal stability of the films. However, the mechanical properties of the films decreased. Meanwhile, the film also has excellent antibacterial efficiency (more than 99.99%) and antioxidant activity, as well as good storage stability. The eugenol can be released from the film to prolong the shelf life of the meat product without affecting the color, texture, and sensory quality of the meat.

Du[25] et al. studied the application of *Lactobacillus plantarum* capsules and eugenol as potential biological control agents in apple slices. *Lactobacillus plantarum* capsules can release probiotics, which can compete with other bacteria for nutrients to prevent the growth of foodborne pathogens and spoilage microorganisms. The fresh-cut apples were protected from food-borne pathogens, inhibited enzymatic browning, and kept the freshness of fresh-cut foods.

Wang Lili[26] et al. Studied the preparation of core-shell EG-emulsion/PEO-CS film by coaxial electrospinning with chitosan (CS) as the shell, eugenol (EG) emulsion as the sustained-release system, and polyethylene oxide (PEO) as the core material. *Pseudomonas fluorescens* was used to study the EG-emulsion/PEO-CS film. The film had good thermal stability and eugenol had good slow-release performance, which could be used in the field of food preservation.

The application of eugenol in the field of food fresh-keeping packaging is gradually expanding. Because of its superior natural activity, more and more food packaging combined with eugenol is modified and adjusted accordingly to further meet people's demand for healthy food. However, different food processing methods are different, and it is still a challenge to extend the shelf life by controlling the slow-release performance of eugenol under different conditions on the premise of ensuring the freshness, color, and quality of food.

4.2 Field of Antifouling and Antibacterial Coating

Marine biological pollution refers to the process by which marine organisms adhere to underwater surfaces such as ship hulls, pipelines, oil surfaces, and offshore sensors. This phenomenon has caused a great economic burden on the maritime industry. For a long time, the development of environmentally friendly and long-term marine antifouling coatings has been a great challenge[27] for the shipping industry. The traditional self-polishing antifouling coatings are mainly based on the release of bactericides to eliminate the microorganisms on the surface of the coating during the process of hydrolysis polishing. Its advantages are high efficiency and easy application, but it will hurt the marine ecological environment. New environmentally friendly coatings have become the current research trend, and eugenol has been widely used in the field of marine antifouling because of its antibacterial property.

Sha[28] et al. First introduced quaternary ammonium salt component into butyl resin-poly eugenol methacrylate loxyethyl trimethyl ammonium chloride-hexafluoro-butyl methacrylate-methyl methacrylate-butyl methacrylate-ethylene glycol methyl ether methyl acrylate (EMQFP) coating by one-step radical polymerization method. The eugenol produced by hydrolysis can be anchored on the quaternary ammonium salt on the surface of the coating for a while and will not be immediately released into the seawater. The coating can improve the utilization rate of eugenol and effectively enhance the antifouling effect of the coating in the ocean.

Yu[29] et al. prepared a self-polishing antifouling coating polymer based on methacrylic acid eugenol. The hydrolysis of the phenolic ester group realizes the transformation of the resin segment from hydrophobicity to hydrophilicity, and the self-polishing process can release the natural and non-toxic eugenol into seawater, and the release rate is very stable, so the coating has good antibacterial performance and anti-algae performance.

Antifouling coatings based on the antibacterial properties of eugenol can effectively solve the problem of marine biological pollution without polluting the environment and reduce the

corresponding economic losses. To maintain a stable release rate of eugenol during shipping, it is necessary to find a suitable component to anchor eugenol on the hull coating. Further efforts are needed to ensure the safety of the hull and equipment throughout sea transportation.

4.3 Prosthodontics

Periodontitis is a common oral disease, which is a chronic infection of four kinds of periodontal supporting tissues (gingiva, periodontal ligament, alveolar bone, and cementum). It often leads to inflammatory destruction of periodontal supporting tissues, and severe periodontitis can even lead to tooth loss. The main culprit is periodontal disease pathogens. Eugenol, as a natural active antibacterial substance, has shown strong activity against oral anaerobic bacteria, such as *Prevotella intermedia*, *Fusobacterium nucleated*, and *Streptococcus mutant*.

Zhang[30] et al. studied the inhibitory effect of clove leaf essential oil on oral anaerobic bacteria *Porphyromonas gingivitis*. The main active substance of clove leaf essential oil is eugenol, which has bacteriostatic activity against *Porphyromonas gingivitis* at a concentration of 31.25 μM . Eugenol can penetrate the cell membrane and irreversibly destroy the integrity of the plasma membrane. Inhibit biofilm formation in the initial stage.

Mosquera[31] et al. developed a solvent-free material with antibacterial properties for use as dental floss by incorporating eugenol into polyamide fibers using a supercritical CO₂-assisted impregnation method. The study of mechanical properties shows that the Young's modulus of the material is reduced and the tensile strength is increased. At the same time, the release kinetics and bacteriostatic activity of the impregnated dental floss on eugenol in air and artificial saliva under atmospheric pressure were studied, and it was found that the bacteriostatic activity of the impregnated dental floss on *Escherichia coli* and *Staphylococcus aureus* was as high as 99.99%, which was excellent when used as dental floss.

Oral disease is considered a major public health problem because of its high incidence worldwide. People are more willing to treat oral disease than to develop oral habits (brushing teeth on time, using dental floss) to prevent disease. Eugenol plays an important role in both the prevention and treatment of oral disease. In the future, eugenol will be used to prepare related drugs, which is expected to further protect people's oral health.

4.4 Poultry Production Area

Poultry production is one of the fastest-growing animal husbandry industries. Because of its advantages such as the least land use, the fastest production speed, and the convenience of feed technology progress, the global per capita consumption of poultry meat has been increasing. The growing trend of poultry meat consumption has increased the attention of relevant professionals to this industry, and consumer awareness and concern about the safety risks of this industry have also increased. Bacteria and other microorganisms in live poultry have become a huge challenge for the industry, and eugenol can solve the related problems for the industry because of its natural antibacterial activity.

Murat[32] et al. studied the inhibitory activity of eugenol against *Clostridium jejunity*, the most common bacteria causing gastroenteritis, which is a major cause of foodborne mortality. Eugenol has a high inhibitory activity on *Clostridium jejunity*, which can effectively solve the hygienic problem of *Clostridium jejunity* contaminated poultry and ensure the safety of poultry at all stages of the food chain from farm to table.

Nunes[33] et al. studied the effect of eugenol on bovine mastitis, which is an infectious disease affecting the mammary gland of dairy cows and has considerable economic losses. The culprit of bovine mastitis is *Staphylococcus aureus*. Eugenol has a good inhibitory effect on *Staphylococcus aureus*, and it is a good measure to use eugenol to treat bovine mastitis.

Eugenol has antibacterial activity on a variety of food-borne microorganisms and has the potential to improve the safety of poultry meat. At present, eugenol is more used in the safety and health of poultry, which can effectively alleviate consumer concerns about the safety of poultry meat. In addition, the

mechanism of eugenol on the intestinal health of poultry needs to be further clarified. Dig deep into the impact[34]of eugenol on intestinal flora and contribute to the field of poultry production.

4.5 Agricultural Sector

Today, with the rising price of pesticide raw materials, people's demand for pesticides is also increasing. Researchers must explore pesticide raw materials in depth, and further development of plant-based pesticides is the trend for future pesticides. Plant clove can become the raw material source of plant pesticides, or extract the corresponding components from the plant as the additive of plant pesticides.

Yu Weibo[35]studied the inhibitory effect of eugenol on *Panax notoginseng* root rot fungi, and found that it had a good inhibitory effect on fungi isolated from susceptible plants of *Panax notoginseng* root rot, could inhibit the activity of fungi-related enzymes, change the gene transcription level of fungi, and also had a certain control effect on pathogenic potted plants. But also can improve the quality of the *Panax notoginseng* during storage.

Nurmansyah[36]et al. studied the effect of clove essential oil (eugenol, the main component) as an additive on plant pesticide thorn flowers, and found that eugenol had a synergistic effect on thorn flowers, the bacteriostatic activity of thorn flowers was enhanced, and eugenol could inhibit the metabolic process of fungi, thus interfering with the growth of pathogenic fungi.

Eugenol can be used as a source of plant-based pesticides or used as an additive to be compounded with pesticides to synthesize a new formula, and eugenol exerts superior antibacterial performance in two ways, and eugenol is low in price and higher in safety so that development of plant-based pesticides by using eugenol can not only reduce the cost of pesticides but also improve the safety factor of people.

5. Summary and Outlook

Eugenol comes from nature, is cheap and easy to get, and has antioxidant, anticancer, analgesic and anti-inflammatory, antibacterial, and other activities. These biological activities can be comprehensively utilized by appropriate methods to meet the application needs of natural active substances in different fields, among which the well-known antibacterial activity has been promoted in many fields. It can meet people's high demand for food safety, bring new hope to the maritime industry, be widely used in dental prevention and treatment, and shine in the field of poultry production and pharmaceutical preparations.

Eugenol has the advantages of high safety and high bioavailability and has become a research hotspot in the field of antibacterial in recent years. However, low doses of natural botanical compounds may not achieve a satisfactory antibacterial effect, and high doses will increase the cost and limit the promotion and application of natural botanical compounds. And eugenol is a lipophilic essential oil, the main mechanism of this substance acting on bacteria to kill them is the permeability of eugenol to bacterial membranes, but this mechanism is also a limitation of the antibacterial function of eugenol. Therefore, it is a challenge in the future to develop the compound of eugenol and other substances to achieve synergistic antibacterial effect, to achieve the antibacterial effect of "1 + 1 > 2", to find a suitable nanoparticle carrier loaded with eugenol and its compound substances, to improve the permeability of eugenol to biofilm, to break the antibacterial limitation of Eugenol, and to achieve synergistic antibacterial effect with other substances.

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