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Review Article

Garlic and Cloves as Promising Antibacterial Agents Against Cariogenic Bacteria- A Mini Review

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Abstract:

Dental caries is the most common noncommunicable disease in the world. Cariogenic bacteria in the oral cavity, such as *Streptococcus mutans* and *Lactobacillus acidophilus*, cause acid formation, demineralization, and tooth damage by metabolizing carbohydrates. However, many antibacterial agents used in dental caries cause several side effects such as nephritis, eosinophilia and hemolytic anemia, hence it is important to search for some natural-based remedies for the treatment dental caries. Garlic (*Allium sativum*) is one of the most widely investigated therapeutic plants. It exhibits a wide range of antibacterial activity against both gram-positive and gram-negative bacteria. Due to the presence of a variety of organosulfur chemicals and biologically active constituents including allicin, allicinase, diallylsulphide etc. Clove (*Syzygium aromaticum)* is widely used in the medicinal, fragrance, and flavoring sectors due to its broad variety of pharmacological and biological effects. The essential oil extracted from clove is used in dentistry as it has the ability to relieve pain. Mainly due to the presence of eugenol. The essential oil of cloves exhibited strong antibacterial activity against cariogenic bacteria via the destruction of cell walls and membranes, followed by the loss of essential intracellular components, which ultimately leads to bacterial death. The present review mainly aims to discuss the potential ability of garlic (*Allium sativum*) and cloves (*Syzygium aromaticum)* to activate different antibacterial mechanisms against cariogenic bacteria.

Key words: Cariogenic bacteria, Garlic (*Allium sativum*), Clove (*Syzygium aromaticum),*

1. Introduction

More than 750 species of bacteria live in the oral cavity. Among them, a significant number of bacteria cause oral diseases. Cariogenic bacteria are the major agents of dental caries. They attack dental enamel by converting sugar and starch into acids that remove calcium from the enamel, while living in biofilm. Examples are *Streptococcus mutans, Streptococcus sobrinus* , *Lactobacillus acidophilus.* Many antibacterial agents, including cetylpyridinium chloride, chlorhexidine, and amine uorides, as well as products containing these agents, have been utilized in the prevention and treatment of oral diseases. These substances have been demonstrated to be harmful to oral tissues and overall health and to discolor teeth. Resistance of pathogenic bacteria to antibiotics and chemotherapy has increased the global need for alternations [1]. In recent years, the ability to prevent the adhesion of cariogenic bacteria without compromising the important health benefits of the local oral microbiota has generated renewed interest in the antimicrobial qualities of several natural products. Natural phytochemicals have great potential for use as adjunctive therapeutic agents against cariogenic microbes in the prevention and treatment of oral caries. Phytochemicals that inhibit mineralization are among the other cariostatic processes for natural products that have been identified [1].

Nowadays, traditional drugs are the primary source of healthcare for 80% of the world's population. Extraction of natural plants and their constituents exhibit a variety of biological actions, including bactericidal, fungicidal, anti-inflammatory, analgesic, sedative, spasmolytic, and local anaesthetic properties [1].

Garlic (*Allium sativum*) is one of the most widely investigated therapeutic plants [2]. The antibacterial effects of garlic have been known for centuries, with many of its therapeutic characteristics originally referenced in an Egyptian recipe called Papirus Ebers circa 1500 BC. It is currently utilized in folk medicine to cure many different illnesses [3]. Garlic formulations contain a variety of organosulfur chemicals, including N-acetylcysteine (NAC) and S-allyl-cysteine (SAC) and biologically active constituents. They are allicin, allicinase, diallylsulphide and allymethyltrisulphide [3].

Allicin and garlic extract have been proven to exhibit antibacterial action against a wide range of bacteria, including *Escherichia*, *Salmonella*, *Staphylococcus*, and *Streptococcus* [4]. Sulphur-containing chemicals have been discovered in garlic cloves by chemical analysis. The positive antibacterial effects of garlic are due to the presence of sulphur-based compounds, in which allicin and its breakdown products, diallyl sulphide (DAS) and diallyl disulfides are the most important. *Streptococcus mutans* is known to be affected by sulphur compounds [5]. Specific oral *streptococci* and *lactobacilli* have been proven to be sensitive to garlic extract, and a mouthwash containing garlic extract was more effective at decreasing total salivary bacterial count and *streptococcal mutans* count. This suggests that garlic extract has antibacterial properties and can be used as an effective oral hygiene aid [4].

Clove (*Syzygium aromaticum L. Myrtaceae*) is an aromatic plant high in volatile chemicals and antioxidants including eugenol, -caryophyllene, and -humulene. Clove essential oil includes a high concentration of phenolic compounds, which have a variety of biological actions such as antibacterial, antifungal, insecticidal, and antioxidant characteristics [6]. The antibacterial and antioxidant activities of the oil are due to its main component, eugenol (2-methoxy-4-allylphenol). It also has pro-oxidant and antioxidant properties and is a flavoring ingredient in food and cosmetic products [7].

Therefore, the present review mainly focuses on the potency of garlic and cloves against cariogenic bacteria. This review will hereby provide the evidence necessary to conduct my research related to the antibacterial activity of a mixture of garlic and clove extracts against cariogenic bacteria and also to the development of a herbal mouthwash using these two extracts.

2. Cariogenic bacteria.

The oral cavity contains around 700 different microbial species, that maintain a dynamic balance in it. Tooth decay caused by oral microbial flora is one of the most frequent and prevalent infectious disorders in humans. The balance and interaction of cariogenic and non-cariogenic microorganism populations are essential for the development of tooth decay [8]. Most cariogenic bacteria are acidogenic and acid-uric. *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacilli* are related aciduric bacteria [9]. *Streptococcus mutans* is the leading cariogenic pathogens in tooth decay. *Mutans* exhibit several characteristics that may be essential in cariogenesis, such as the formation of sucrose-dependent biofilms, relatively high acidity, and high acidogenesis) [9]. Removing plaque and reducing cariogenic bacteria are key to preventing tooth decay.Additionally, it has been demonstrated that some oral streptococci and lactobacilli are susceptible to garlic extract, and studies have indicated that mouthwashes containing garlic extract are more successful at lowering both the total number of bacteria in saliva and the number of *mutans streptococci* [4].

3.Targets (Mechanisms) of anti-microbial therapy.

The potential of bacteria to acquire resistance to antimicrobial agents makes infections difficult to control. The primary mode of action of antimicrobial medicines used to treat bacterial infections are varied. They include interference with cell wall synthesis, depolarizing the cell membrane, inhibition of protein ,nucleic acid synthesis, inhibition of metabolic pathways, inhibition of membrane function, and inhibition of ATP Synthase [10].

3.1. Interference with cell wall synthesis.

Cell walls consist of peptidoglycans that enclose bacterial cells [11]. The formation of peptidoglycan, the outermost layer and key ingredient of the cell wall, is vital for the structure's stability. Certain antibiotics interfere with the synthesis of peptidoglycans, compromising the integrity of the cell wall [10].The peptidoglycan's glycan strands are linked together by transglycosidases, and particular peptide cross-linkages are created by extending peptide chains from the polymers' sugars. In the presence of penicillin-binding proteins (PBPs), glycine residues crosslink the D-alanyl-alanine component of the peptide chain. This cross-linking increases the cell wall [12].

Antibiotics include glycopeptides and beta-lactams that prevent the production of peptidoglycans and make cells more vulnerable to osmotic pressure and the autolytic process. [13]. Therefore, natural extracts that can prevent the formation of a component of the bacterial cell wall can be beneficial in controlling bacterial infections.

3.2. Depolarization of bacterial membrane.

Membranes that can process information and signals are used by bacteria [14]. By depolarizing the membrane potential, bacteria can develop a "persister" condition where they are more vulnerable to medication. Proton pumps, which depend on the membrane potential to maintain pH homeostasis, maintain the "persister membrane potential" (PMF). Cell division, which requires the potential of the membrane for the proper localization of the division site, can be prevented by native protonophores. The dynamic response of the bacterial membrane potential to molecular and electrochemical stimuli also permit interaction between cells [15]. In order to relieve bacterial infections, natural extracts that can depolarize the cell membrane are effective.

3.3. Inhibition of protein synthesis

Protein synthesis is a complicated, multi-step process requiring numerous enzymes and conformational alignment. The majority of antibiotics, however, interfere with the 30S or 50S subunits of the 70S bacterial ribosome, one can prevent the synthesis of bacterial proteins [10]. Tetracyclines, such as doxycycline, inhibit the interaction between aminoacyl-tRNA by blocking the 30S ribosome's A (aminoacyl) site. They are capable of inhibiting protein synthesis in eukaryotic (70S and 80S) ribosomes

[12].Therefore, natural extracts that have the ability to alter the 30S and 50S ribosomal units' structural integrity can be thought of as a successful treatment target for bacterial infections.

3.4. Interference with nucleic acid synthesis.

Quinolones, a significant class of antibiotics, prevent topoisomerases, most notably topoisomerase II (DNA gyrase), an enzyme required for DNA replication, from functioning normally. This prevents the synthesis of DNA [12]. DNA gyrase generates small breaks in closed-circular DNA superhelical twists, relaxes supercoiled DNA molecules, and re-joins phosphodiester links. The DNA strands can then be replicated by DNA or RNA polymerases as a result. Norfloxacin, levofloxacin, and ciprofloxacin are a few examples of fluoroquinolones, second-generation quinolones that are efficient against both gram-negative and gram-positive bacteria [12].

3.5. Inhibition of a metabolic pathway.

The routes by which bacteria produce nucleic acids and amino acids are the focus of antibiotics classified as bacterial metabolism inhibitors.Tetrahydrofolic acid (TH4), an important coenzyme, is used by all living things to produce nucleic acids and certain amino acids. Acid PABA (para-aminobenzoic) bacteria use PABA, which serves as a precursor, to produce folic acid.By interfering with TH4 production, bacterial metabolism inhibitors affect bacterial metabolic pathways[12].

4. Bioactive compounds present in Garlic (*Allium sativum*) and cloves (*Syzygium aromaticum*).

Figure 1- Garlic ***(Allium sativum*).**

4.1 Bioactive compounds present in Garlic (*Allium sativum*).

The old English word garleac, which means spear leek, is where the name "garlic" originates. The words 'gar' and 'leac' both allude to spears, namely spear-shaped leaves. Garlic (Figure 1) has been around for 5000–6000 years. Although it is endemic to central Asia, it is challenging to identify the nation where it first appeared [16]. It is recognized as the second most widespread Allium species after onion. The distinctive flavor of garlic cloves, which is the consequence of intricate biochemical interactions, is the primary characteristic of garlic products [17].

Among all the herbal medicines, garlic is one of the oldest plants used in medicine [18]. And it is one of the most useful aromatic spices. It is botanically known as *Allium sativum* L.[16]. Garlic has a calorie value of 140 and a 100g serving contains 63.8g of water, 5.3g of protein, 28.2g of carbohydrate, 0.2g of oil, and 11g of cellulose ,Garlic can be consumed raw, and there is also tablets, capsules, and extracts obtainable [19].

The taste and odor of the garlic come from allicin [S-(2-propenyl)-2-propene-1-sulfinothioate], which is the sulfur-containing molecule that is most physiologically active in garlic. Organosulfur compounds, including alliin, allicin, and diallyl sulfide are responsible for the defense oxidative damage. An enzyme known as alline- lyase catalyzes the transformation of alliin, an amino acid, into allicin when the bulbs are crushed [3].

The parent compound was alliin (Figure 2) and gamma-glutamyl S-allylcysteine, which is transformed into S-allylcysteine (SAC), S-allylmercaptocysteine (SAMC), and other allyl sulfur compounds. The water-soluble sulfur compounds of garlic may occur, especially after fermentation in alcohol [18]. It is possible for allicin, a lipid-soluble sulphur molecule, to induce intolerance, allergic reactions, and digestive issues. Allicin can be swiftly eliminated by boiling [1]. Allicin is further transformed into the antimicrobial compound diallyldisulphide once it is exposed to air, and the reduction of cysteine results in the breaking of disulphide bond in microbial proteins. Allicin and thiosulphonates found inside garlic are primarily responsible for the bactericidal and bacterial growth-inhibiting effects [3].

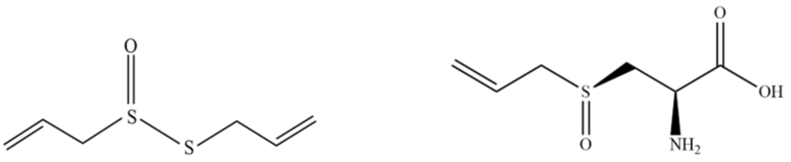

Figure 2- The chemical composition of primary organosulfur components of garlic.

4.2. Bioactive compounds present in cloves (*Syzygium aromaticum)*

The clove, also known as *Syzygium aromaticum* (Figure 3) is a tropical perennial. It serves as a source of essential oils that is frequently used in cosmetics and medicine [7]. Clove essential oil can be made from the buds, leaves, and stem of the clove plant, all of which differ in color, flavor, and chemical composition. Buds are where the best clove oil is produced. The composition of essential oils can vary depending on genetic variables, climate conditions, and method of production. [20]. In addition, clove is rich in minerals such as , sodium iron, phosphorus, calcium, potassium , vitamin A and vitamin C [21].

Figure 3- Cloves (*Syzygium aromaticum)*

In addition, several phytochemicals such as eugenol, eugenyl acetate, beta-caryophyllene, cinnamaldehyde, 2-heptanone, thymol, methyl salicylate, alpha- humulene, gallic acid, ellagic acid and oleanolic acid have been identified in clove essential oil [22].

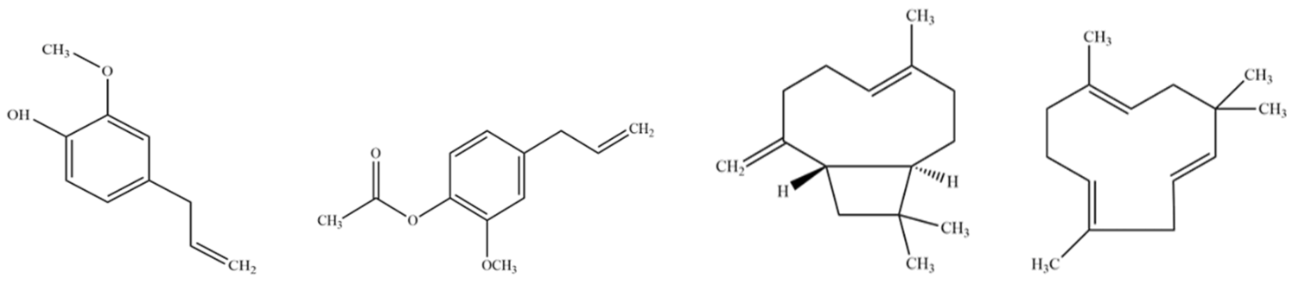

Figure 4- The chemical structure of the main compounds of cloves.

Eugenol (4-allyl-2-ethoxyphenol) (Figure 4) is the main compound of clove essential oil. It is easily soluble in organic solvents and slightly soluble in water [23].Eugenol is the main chemical compound that is mostly responsible for clove odor, and it makes up between 72- 90% of the essential oil produced from cloves [24]. Eugenol is a volatile substance that ranges in color from colorless to light yellow. It has an intense odor and flavor. Biological effects of eugenol have affected insecticidal, antibacterial, anti-inflammatory, wound-healing, antiviral, antioxidant, and anticancer actions. Similar to the effects of local medications including lidocaine, eugenol causes the transient receptor potential cation channel V1 (TRPV1) to become activated [6].

A eugenol derivative known as eugenyl acetate has antibacterial, anticancer, antimutagenic, antioxidant, and antivirulence properties. It has been described as a potent antioxidant agent, At 35 g/mL, it demonstrated 90.30% DPPH free radical scavenging, while at 60 g/mL, it demonstrated 89.30% NO free radical scavenging [6].

Clove contains a sesquiterpene called caryophyllene. β-Caryophyllene is soluble in ethanol but is insoluble in water. It has demonstrated evidence of having antibacterial, anticancer, anti-inflammatory, antioxidant, anxiolytic-like, and local anaesthetic actions as well as anticancer activities, including those against breast, prostate, skin, pancreatic, lymphatic, leukemia, and cervical cancer. Caryophyllene's

beta-chemo sensitizing characteristics allow drugs more effective against tumor cells [6].
The anti-inflammatory and anticancer properties of this substance have been demonstrated in colon, lung, prostate, and breast cancer. It prevents the CYP3A enzyme from metabolizing drugs in the liver microsomes of rats and humans. In model mice and rats, oral treatment of -humulene and -caryophyllene (50 mg/kg) resulted in anti-inflammatory effects that were comparable to those of dexamethasone [6].

5. Antibacterial effect of garlic *(Allium sativum)* and cloves (*Syzygium aromaticum).*

5.1. Antibacterial activity of garlic *(Allium sativum).*

Allicin present in garlic is declared to be an antibacterial agent. It exhibited activity against more gram-negative and gram-positive bacteria like *Helicobacter pylori, Escherichia coli, Streptococcus mutans, Lactobacillus casei*. Allicin has also shown efficacy against methicillin- resistant *S.aureus* [1]. Under natural conditions, allicin is a protein oxidizer that breaks down cysteine or glutathione residues. Allicin's antimicrobial effect is principally caused by its chemical interactions with thiol-containing enzymes such as alcohol dehydrogenase, RNA polymerase, and thioredoxin reductase. Allicin is a dose-dependent biocide that can disrupt the metabolism of cystine proteinase and kill all eukaryotic cells as it reacts with the thiol groups found in all living cells [25].

The antimicrobial effects of garlic are frequently attributed to allicin. Allicin is widely known for its capacity to modify sulfhydryls and prevent sulfhydryl enzyme activity. Raw garlic doesn't contain allicin. Allinase enzyme present in garlic degrades alliin and form allicin. Antimicrobial action of allicin is caused by contact with thiol-containing enzymes, such as cysteine proteases and alcohol dehydrogenases. It reacts with free thiol groups relatively quickly via thiol-disulphide exchange. It has been hypothesized that the development of resistance to allicin arises 1000-fold less readily than it does to specific antibiotics because these enzymes frequently serve as an essential part of bacterial nutrition and metabolism [25].

In addition, sulphur found in garlic and bioflavonoids like quercetin and cyanidin are considered to be extremely effective at preventing away illnesses and infections. It has been discovered that the active ingredients of garlic, such as allistatin I and allistatin II, are effective agents against bacteria such as *Staphylococcus* and E. coli [26]. The antibacterial spectrum of different garlic species exhibited different levels of activity due to the variation of bioactive compounds [26]. Various garlic extracts, chloroform, methanolic including aqueous, and ethanolic extracts have been demonstrated to inhibit a range of harmful bacteria, with varying degrees of susceptibility. In one case, a study concluded that ethanolic garlic extract had a higher level of inhibitory impact than aqueous extract against E. coli and *Salmonella typhi,* which had little to no inhibitory effect [1]. Meanwhile, methanolic garlic extract had antibacterial effects against each type that was tested, including gram-positive strains like *Bacillus subtilis* and *S. aureus* as well as gram-negative strains like *Klebsiella pneumoniae.* But none of the extracts from hexane, ethyl acetate, or chloroform had any antibacterial properties. Further, the garlic extracts prevent the growth of pathogenic intestinal bacteria, the main cause of diarrhea in both people and animals and enterotoxigenic E coli strains. Garlic has been shown to have antibacterial properties as well as the ability to suppress toxins created by bacterial infections [1].

5.2 Anti-bacterial activity of cloves

In a study by dormans and deans, six essential oils were examined for their antibacterial effects on 25 different bacterial species. All of the bacteria tested varied in their sensitivity to the essential oils. Thyme, oregano, and clove oils had higher activity levels [27].

Cloves essential oil (CEO) has shown broad-spectrum inhibitory activity against pathogens. It can inhibit gram-negative bacteria and gram-positive bacteria. The -OH groups in the meta and ortho locations of the primary chemical compositions have been correlated with the antibacterial activity. These -OH groups can interact with microbial cell cytoplasmic membranes. Because of its lipophilic characteristics, CEO is capable of passing through the cell membrane. CEO interacts with polysaccharides, fatty acids, and phospholipids in a way that compromises the integrity of cellular membranes, allows for the leakage of cellular contents, and interferes with proton pump function, all of which result in cellular death. CEO inhibits gram-positive bacteria more than gram-negative bacteria. Gram-positive bacteria have a mucopeptide layer that is diffusible and makes them vulnerable to antimicrobial treatments. On the other hand, gram-negative bacteria's lipopolysaccharide complex layer in the outer cell membrane can greatly slow the pace at which lipophilic antibacterial chemicals diffuse through the cell membrane [6].

Another study confirmed that the eugenol extracted from clove buds was most effective component of the antibacterial action against *Staphylococcus aureus.* Meanwhile, results indicated that the antibacterial action of clove essential oil was dose-dependent [28].

The presence of a free hydroxyl group in the molecule of eugenol is responsible for its antibacterial properties. Researchers have theorized that the hydroxyl group of eugenols would bind to proteins and inhibit enzyme activity. Numerous mechanisms have been proposed to explain whether eugenol affects bacterial cells. This may primarily affect ions and ATP transport by rupturing the cytoplasmatic membrane, which increases nonspecific membrane permeability [29].

The release of K^+ ions from *Listeria monocytogenes* exposed to eugenol confirmed the concept that the cytoplasmic membrane is a target for eugenol activity. The findings showed that eugenol made *L. monocytogenes* cells more permeable to the K+ ion, and that adding eugenol at bactericidal concentrations significantly increased the amount of K+ released into the phosphate-buffered saline (PBS) buffer [29]. According to a study on the morphological and ultra-structural changes in eugenol-treated *Streptococcus agalactiae* cells, after 5 hours of incubation with an inhibitory concentration of eugenol, the planktonic cells of *Streptococcus agalactiae* exhibited a variety of alterations, including changes in cell morphology and disruption of the cell wall [29].

Conclusions

The present review mainly focused on the bioactive compounds present in garlic *(Allium sativum)* and cloves *(Syzygium aromaticum)* and how these bioactive compounds are potent against cariogenic bacteria. According to the review, organosulfur compounds present in garlic and eugenol in cloves are mainly responsible for potent antibacterial activity against a wide range of cariogenic bacteria mainly by disrupting the cell wall and inhibiting protein synthesis. Therefore, it is warranted to determine the synergistic antibacterial effect of the garlic and clove extracts in future studies.

References

[1] E.-S. Batiha *et al.*, "Chemical Constituents and Pharmacological," *Nutrients*, vol. 12, no. 3, p. 872, 2020.

[2] K. B. D. D. S. Ms, M. Shari, and T. K. Dds, "Ef ¿ cacy of Different Concentrations of Garlic Extract in Reduc- tion of Oral Salivary Microorganisms," vol. 15, no. 2, pp. 2011–2013, 2012.

[3] F. C. Groppo, J. C. Ramacciato, R. H. Motta, P. M. Ferraresi, and A. Sartoratto, "Antimicrobial activity of garlic against oral streptococci.," *Int. J. Dent. Hyg.*, vol. 5, no. 2, pp. 109–115, 2007, doi: 10.1111/j.1601-5037.2007.00230.x.

[4] I. M. Bakri and C. W. I. Douglas, "Inhibitory effect of garlic extract on oral bacteria," *Arch. Oral Biol.*, vol. 50, no. 7, pp. 645–651, 2005, doi: 10.1016/j.archoralbio.2004.12.002.

[5] S. Kudva, S. Prabhakar, V. Pai, and A. Tegginamani, "Effects of garlic extract on salivary pH: a clinical study," *Orofac. Sci. J. Sch. Dent. Sci. Univ. Sains Malaysia Arch Orofac Sci*, vol. 7, no. 1, pp. 1–8, 2012.

[6] J. N. Haro-González, G. A. Castillo-Herrera, M. Martínez-Velázquez, and H. Espinosa-Andrews, "Clove essential oil (Syzygium aromaticum l. myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health," *Molecules*, vol. 26, no. 21, 2021, doi: 10.3390/molecules26216387.

[7] C. Jianu, C. Mişcă, D. Stoin, G. Bujancă, and L. G. A. Teodora, "Chemical composition and antioxidant properties of dill essential oil," *Int. Multidiscip. Sci. GeoConference Surv. Geol. Min. Ecol. Manag. SGEM*, vol. 18, no. 6.4, pp. 87–94, 2018, doi: 10.5593/sgem2018V/6.4/S08.012.

[8] Z. Tong, L. Zhou, J. Li, R. Kuang, Y. Lin, and L. Ni, "An in vitro investigation of Lactococcus lactis antagonizing cariogenic bacterium Streptococcus mutans," *Arch. Oral Biol.*, vol. 57, no. 4, pp. 376–382, 2012, doi: 10.1016/j.archoralbio.2011.10.003.

[9] H. K. Kuramitsu and B. Wang, "Virulence properties of cariogenic bacteria," vol. 4, pp. 4–7, 2006, doi: 10.1186/1472-6831-6-S1-S11.

[10] R. P. Lange, H. H. Locher, P. C. Wyss, and R. L. Then, "The Targets of Currently Used Antibacterial Agents: Lessons for Drug Discovery," *Front. Med. Chem.*, pp. 62–96, 2012, doi: 10.2174/9781608054640113060006.

[11] P. A. Levin and E. R. Angert, "Small but mighty: Cell size and bacteria," *Cold Spring Harb. Perspect. Biol.*, vol. 7, no. 7, pp. 1–11, 2015, doi: 10.1101/cshperspect.a019216.

[12] K. Upadhya R, L. Shenoy, and R. Venkateswaran, "Effect of intravenous dexmedetomidine administered as bolus or as bolus-plus-infusion on subarachnoid anesthesia with hyperbaric bupivacaine," *J. Anaesthesiol. Clin. Pharmacol.*, vol. 34, no. 3, pp. 46–50, 2018, doi: 10.4103/joacp.JOACP.

[13] K. Bush and P. A. Bradford, "b -Lactams and b - Lactamase Inhibitors: An Overview," no. Table 1, 2016.

[14] A. Giedraitienė, A. Giedraitienė, A. Vitkauskienė, R. Naginienė, and A. Pavilonis, "Correspondence to Antibiotic Resistance Mechanisms of Clinically Important Bacteria," *Rev. Med.*, vol. 47, no. 3, pp. 137–183, 2011.

[15] J. M. Benarroch and M. Asally, "The Microbiologist's Guide to Membrane Potential Dynamics," *Trends Microbiol.*, vol. 28, no. 4, pp. 304–314, 2020, doi: 10.1016/j.tim.2019.12.008.

[16] M. M. Kshirsagar, A. S. Dodamani, G. N. Karibasappa, P. K. Vishwakarma, and J. B. Vathar, "Antibacterial activity of garlic extract on cariogenic bacteria : An in vitro study," pp. 165–168, 2019, doi: 10.4103/ayu.AYU.

[17] N. Martins, S. Petropoulos, and I. C. F. R. Ferreira, "Chemical composition and bioactive compounds of garlic (Allium sativum L.) as affected by pre- and post-harvest conditions: A review," *Food Chem.*,

vol. 211, pp. 41–50, 2016, doi: 10.1016/j.foodchem.2016.05.029.

[18] R. Z. Chekki, A. Snoussi, I. Hamrouni, and N. Bouzouita, "Chemical composition , antibacterial and antioxidant activities of Tunisian garlic (Allium sativum) essential oil and ethanol extract," vol. 3, no. 4, pp. 947–956, 2014.

[19] G. Goncagul and E. Ayaz, "Antimicrobial effect of garlic (Allium sativum) and traditional medicine," *J. Anim. Vet. Adv.*, vol. 9, no. 1, pp. 1–4, 2010, doi: 10.3923/javaa.2010.1.4.

[20] K. Kaur, S. Kaushal, and R. Rani, "Chemical Composition, Antioxidant and Antifungal Potential of Clove (Syzygium aromaticum) Essential Oil, its Major Compound and its Derivatives," *J. Essent. Oil-Bearing Plants*, vol. 22, no. 5, pp. 1195–1217, 2019, doi: 10.1080/0972060X.2019.1688689.

[21] J. S. Packyanathan and G. Prakasam, "Antibacterial effect of clove oil against clinical strains of Escherichia coli," *J. Pharm. Sci. Res.*, vol. 9, no. 7, pp. 1203–1204, 2017.

[22] M. I. Nassar *et al.*, "Chemical constituents of clove (Syzygium aromaticum , Fam . Myrtaceae) and their antioxidant activity," *Rev. Latinoam. Quim.*, vol. 35, no. 3, pp. 47–57, 2007.

[23] N. Nurdjannah and N. Bermawie, "Cloves," *Handb. Herbs Spices Second Ed.*, vol. 1, pp. 197–215, 2012, doi: 10.1533/9780857095671.197.

[24] M. A. Uddin, M. Shahinuzzaman, M. S. Rana, and Z. Yaakob, "Study Of Chemical Composition And Medicinal Properties Of Volatile Oil From Clove Buds (Eugenia Caryophyllus) | International Journal Of Pharmaceutical Sciences And Research," *Int. J. Pharm. Sci. Res.*, vol. 8, no. February, pp. 895–899, 2017, doi: 10.13040/IJPSR.0975-8232.8(2).895-99.

[25] T. V. R. Rakshanaa and R. V. Geetha, "Evaluation of antimicrobial action of honey on cariogenic bacteria - an in vitro study," *J. Pharm. Sci. Res.*, vol. 9, no. 5, pp. 705–707, 2017, doi: 10.4103/ayu.AYU.

[26] G. Goncagul and E. Ayaz, "Antimicrobial Effect of Garlic (Allium sativum)," pp. 91–93, 2010.

[27] L. Nuñez and M. D'Aquino, "Microbicide activity of clove essential oil (Eugenia Caryophyllata)," *Brazilian J. Microbiol.*, vol. 43, no. 4, pp. 1255–1260, 2012, doi: 10.1590/S1517-83822012000400003.

[28] J. G. Xu, T. Liu, Q. P. Hu, and X. M. Cao, "Chemical composition, antibacterial properties and mechanism of action of essential oil from clove buds against staphylococcus aureus," *Molecules*, vol. 21, no. 9, pp. 1–13, 2016, doi: 10.3390/molecules21091194.

[29] A. Marchese *et al.*, "Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint," *Crit. Rev. Microbiol.*, vol. 43, no. 6, pp. 668–689, 2017, doi: 10.1080/1040841X.2017.1295225.