P- ISSN 1991-8941 E-ISSN 2706-6703 Journal of University of Anbar for Pure Science (JUAPS) Open Access
2024,(18), (01):76 – 85

# Studying the effect of plant extracts on Gram-positive and Gram-negative pathogens: Review

**Alaa S. Sadah Al-Halfi** [*]**, Maher Zaki Faisal Al-Shammari and Suaad Khalil Ibrahim**

Department of Biology, College of Education For Pure Science Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq;

**ARTICLE INFO**

Received: 02 / 11 /2023
Accepted: 03 / 12 / 2023
Available online: 17 / 06 / 2024

DOI:10.37652/juaps.2023.144339.1159

**Keywords: Ginger, Gram-positive and Gram -negative pathogens MDA, Thyme, Virulence genes.**



**ABSTRACT**

Gram-positive and Gram-negative antibiotic-resistant bacteria (called MDA) pose a continuing and ever-increasing threat to human health around the world, especially in poor countries. The aim of the study is to find alternatives to traditional antibiotics or extract effective compounds that work synergistically with or as an alternative to antibiotics. Many studies have proven the ability of plant extracts, especially the volatile oils in aromatic or medicinal plants such as Ginger and Thyme, to inhibit or kill negative bacteria such as *Pseudomonas aeruginosa* and Gram-positive bacteria such as *Staphylococcus aureus*, which is resistant to antibiotics. It was found that these extracts consist of chemical compounds. Carbohydrate acids destroy and dissolve bacterial membranes and destroy the target cell. Bacterial colonies were diagnosed based on their morphological characteristics, such as the size, colour, edges and height of the colonies, their ability to ferment the sugar lactose, and their ability to produce mucus and hemolysin. Diagnosis of isolates using biochemical tests with three main tests. Molecular diagnosis was carried out by performing PCR tests and using VITEK2 technology. The active compounds were extracted from ginger and thyme using various polar organic solvents, and it was found that virulence genes, such as enzymes, toxins, adhesive proteins, and cell surface proteins produced by *S. aureus* and other types of pathogenic bacteria, are responsible for the infections formed in people infected with this disease.

## Introduction

Antimicrobial resistance (AMR) is currently a global problem, with millions of people dying each year as a result of opportunistic or protozoan pathogens of bacterial species that have become resistant due to mechanisms of horizontal gene transfer (HGT) or biofilm formation. This is a multifaceted problem that has a significant and catastrophic impact on everyone, including humans and the environment [1]. According to the World Health Organization, this has led to an estimate that in 2050, 10 million people will die from untreatable infections caused by multidrug-resistant bacteria and ineffective antibiotics [2]. Most recent studies suggest alternatives to antibiotics (such as plant extracts, honey, propolis, prebiotics, probiotics, synbiotics and prebiotics) and combined with drug therapy, can be used as an adjunct to standard treatments well, allowing drug sensitivity of bacteria to develop multidrug resistance.

Recover pathogens, enhance host immunity, and improve clinical efficiency.

Currently, the availability of new and relevant developments in genomics, as well as transcriptomics and proteomics for researches, may lead to the discovery of new antimicrobial drugs and a new generation of antibiotics resulting from non-antibiotic plant extracts. Despite all this, many areas related to natural products and their combination with standard medicines are still not precise and clear [3, 4].

The resistance developed by Gram-positive and Gram-negative bacteria to many drugs has made them difficult to treat and even untreatable with currently available antibiotics. The use of plant extracts such as ginger and thyme extract may work synergistically with some antibiotics to limit the development and spread of multi drug-resistant bacteria [5,6]. It is known that the most types of bacteria resistant to antibiotics are *Staphylococcus aureus*, as well as some species belong to the genus *Bacillus*, which are gram-positive. This is

*Corresponding author at: Department of Biology, College of Education For Pure Science Ibn Al-Haitham, University of Baghdad, Baghdad, Iraq;ORCID:https://orcid.org/0009-0005-1216236X. ;Tel:+9647700056724 :E-mail address: aalaa.sada2102p@ihcoedu.uobaghdad.edu.iq

due to the fact that these bacteria possess virulence genes and other mechanisms that allow them to remain in the host despite the presence of antibiotics, in addition to their ability to adapt through the formation of continuous genetic mutations from In order to get rid of these antibiotics, the types of dangerous bacteria resistant to gram-negative antibiotics are *Klebsiella pneumonia* and *Pseudomonas aeruginosa* [7,8]. These bacteria have a tremendous ability to resist multiple drugs, and this is also due to their possession of properties that enable them to resist antibiotics, such as the possession of a capsule and its ability to carry the formation of the cell membrane, in addition to the virulence genes that are responsible for the high toxicity of these bacteria. The use of aqueous and alcoholic plant extracts such as ginger extract (Figure 1) and thyme extract has become one of the successful solutions to inhibit the growth and reproduction of gram-negative and gram-positive bacteria the action of these extracts is either directly in killing bacteria or works synergistically with antibiotics to kill and inhibit bacteria because they contain many natural chemical compounds such as volatile oils, terpenes, phenols, esters, and antioxidants [9, 10]. In this systematic study, the extent to which these extracts can control certain types of bacteria that cause antibiotic-resistant diseases will be addressed vitality.

The aims of this review is to know the inhibitory effect and the ability of some plant extracts, such as ginger and thyme extract, on specific types of pathogenic bacteria. Identify the feasibility of the synergistic process between antibiotics and plant extracts in inhibiting certain types of Gram-negative and Gram-positive bacteria. What are the properties of the negative and positive antibiotic-resistant bacteria under study and how to suppress and control them specifically through plant extracts?

## Methodology

### Characteristics of Gram-positive bacteria resistant to antibiotics

*Staphylococcus aureus* bacteria are considered a multidrug-resistant (MDR) species, and according to a large group of studies, they are highly resistant to antibiotics and require a long period of recovery, especially isolates transmitted from wounds, diabetic foot, and skin infections. Therefore, strict sterilization procedures must be taken in health institutions to limit transmission infections with these bacteria [11]. It was also found *Bacillus subtilis* bacteria can have virulence genes, as a comparison was made between males and females. The *aspH* gene responsible for producing one of the virulence factors was investigated in a molecular manner using polymerase chain reaction (PCR) technology.

It is also proven that the previously mentioned bacteria, due to their ability to resist multiple drugs, have an arsenal of toxins, virulence genes, and other characteristics such as capsule formation, cell membrane, and adhesion proteins [12, 13].

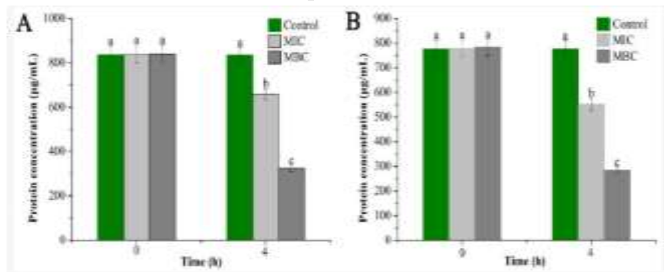


**Figure 1.** Effect of GEO extract on the content of bacterial intracellular proteins of *Klebsiella* (A) and *Staphylococcus aureus* (B). P < 0.05 represents the significant differences shown in the level of probability of error in the form of letters (a, b, c).

As for *Bacillus cereus* bacteria, hemolysin is one of the virulence factors for *B. cereus* bacteria, as this bacteria produces two important types of hemolytic enzymes, namely Cereolysin O. This enzyme is the main responsible for hemolysis in *B. cereus* and is fatal when injected. In mice, Cereolysin AB works synergistically with the Phospolipase C enzyme as a cytolytic unit, which is composed of two enzymes: Phosphatidylcholine - phospholipase and Sphingomyelinase, which work cooperatively to form a double hemolysin called Cereolysin AB, which in turn carries out the process of breaking down red blood cells [14].

### Some types of bacteria that are gram-negative and highly virulent:

In a study conducted at the University of Baghdad, Enteropathogenic *Escherichia coli* (EPEC) bacteria were isolated and identified from children suffering from diarrhea, and then their ability to resist antibiotics and their ability to produce biofilms were revealed. After that, alternative therapeutic methods from natural sources were investigated. Through some traditional plants used in treating diarrhea (*Hibiscus sabdariffa*, *Punica granatum*, and *Citrus aurantifolia*) by studying the inhibitory activity against EPEC bacteria and studying the inhibitory activity on one of the important virulence factors, which is biofilm [15, 16].

Thyme is considered an important medicinal plant, so the effect of protein extracts and essential oils of these seeds has been studied. The plant affects the growth of bacteria that cause urinary tract infections, as six types of bacteria were used, including *Klebsiella* sp. and *Pseudomonas aeruginosa*. *Proteus* and *Staphylococcus aureus* [17]. It was found that the oil extract has an effect in inhibiting the growth of these bacteria, especially at a concentration of 1000 micrograms per ml. The protein extract also showed greater inhibition of bacterial growth in general compared to the Turkish oil extract in particular (1000 mg/ml) recorded the highest growth inhibition against *Proteus* sp. bacteria and the lowest inhibition against *S. aureus* bacteria. The rate of inhibition among other types ranged between 5-20 mm. The study also showed that the effect of the oil extract was that of basic antibiotics against the growth of pathogenic bacteria, close to the effect of the antibiotics used. It was also found that the effect of the protein extract was more effective than the oil extracts compared with the effect of antibiotics, as it was shown in the study that it is possible to use extracts of essential oils and proteins as drug alternatives instead of antibiotics or in combination with them, after conducting detailed clinical studies [18].

**Inhibitory activity of ginger and thyme extract against antibiotic-resistant bacteria:**

Ginger has been used as a medicinal herb to treat infectious diseases, leading to the hypothesis that it may contain chemicals that prevent biofilm formation. To investigate this hypothesis, the ability of ginger to prevent the formation of biofilms of *Pseudomonas aeruginosa* PA14, *Klebsiella* spp. and Staphylococcus *aureus* was evaluated. The persistent biofilm test showed that biofilm development was reduced by 39-56% when ginger extract was added to the culture. In addition, different phenotypes were changed after ginger addition to PA14. Ginger extract reduces the production of extracellular polymeric substances. In addition, ginger extract formed significantly thinner colonies on agar plates containing Congo red. The inhibition of biofilm formation and altered phenotypes appear to be associated with a low level of the second messenger. Importantly, ginger extract inhibits biofilm formation in both Gram-positive bacteria and gram negative. Also, surface biofilm cells formed with ginger extract were more easily detached with the surfactant than those without ginger extract. Taken together, these results provide a basis for the potential discovery of a broad-spectrum biofilm inhibitor [19].

In a study [20], the active substances in the aqueous extract of the ginger plant, *Zingiber officinale*, were identified and their inhibitory effectiveness were tested against Gram-positive and Gram-negative bacterial species, as well as their effect on the phagocytic immune level after they were injected with *Escherichia coli* bacteria into rats dosed with the aqueous extract orally for 8 days at concentrations of 50 and 100 mg/kg of body weight. The results showed that the aqueous extract of ginger contains active groups of tannins, resins, saponins, flavones, phenols, alkaloids, glycosides, and coumarins. As for their effects on bacteria, it was shown that the extract had an inhibitory effect on the inhibitory susceptibility when measuring the inhibitory concentration. The lowest concentration was against the species of *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Pseudomonas aeruginosa*, and its inhibitory effectiveness was confirmed by measuring the diameter of the inhibition zones, which showed that the *E. coli* bacteria were sensitive, as the diameter of the inhibition zone was 20 mm at the concentration of 100 mg/hole, while the most resistant were types of *S. aureus* and *S. pyogenes*, as the diameter of the inhibition zone was 14 mm. In comparison, the extracts were almost equal in effectiveness to the antibiotic ciprofloxacin at concentrations of 0.005 and 0.01 mg/kg.

Ginger has been used because of its antibacterial activity, as studies have shown that the ethanolic extract of ginger has antifungal and antibacterial activities, especially against bacilli, and the extract also reduces. The extract of ginger hexose shows the minimum inhibitory concentration for aminoglycosides in resistant enterococci for vancomycin. It has also been shown that ginger essential oil has an inhibitory effect on *Candida albicans*, *Pseudomonas* sp. and *Bacillus subtilis*, and *Aspergillus niger*, and has an effect against the herpes virus. A protein isolated from ginger rhizome also showed inhibitory activity toward of different fungi such as *Mycosphaerella*, *Fusarium oxysporum*, *Botrytis cinerea Physalospora piricola* and *Arachidicola*, There are many plant extracts that have inhibitory activity against antibiotic-resistant bacteria, including *Eruca sativa* extract [21, 22].

**Active compounds in ginger and thyme:**

The ginger plant (*Zingiber officinale*) has a unique pungent smell, so it can be used as a spice in food. In addition, it contains many biological compounds, such as zingiberene, 6-gingerol, and α-curcumene. Ginger essential oil is a volatile oil that is extracted specifically from the roots. Ginger essential oil (GEO) has potential application in the pharmaceutical, cosmetic and food fields [20]. In 2018, GEO received General Recognition as Safe (GRAS) status by the US Food and Drug Administration (FDA) [23].

In a study [24], ginger extract was found to be antibacterial and anti-biofilm activities of GEO against *S. putrescens* formed by bacteria and its mechanism (Table 1). GEO showed intense antibacterial activity against *S. putrescens* with an MIC and MBC of 2.0 and 4.0 µmol/mL respectively. It turned out that GEO broke down the phospholipids in the membrane, causing the leakage of nucleic acids and protein. XTT and crystal violet assays showed that GEO disrupted biofilms by reducing biomass as well as cellular activity. Both SEM and CLSM images revealed physical injury, significant behavioral changes, and leakage of intracellular components. GEO reduces the amount of biofilms, destroys the biofilm structure, and retards movement. According to these results, GEO has promising potential to be used as a natural food preservative and to promote longer shelf life of foods. Future studies are expected to identify additional mechanisms underlying the anti-biofilm behavior of GEO [13, 15, 24] (Figure 2).

**Table 1.** Some antibacterial compounds and volatile oils found in ginger [25].

| Formula | Compounds | Percentage (%) | Retention Index |
|---|---|---|---|
| $C_{15}H_{24}$ | Zingiberene | 34.4 | 971 |
| $C_{15}H_{22}$ | α-Curcumene | 13.7 | 916 |
| $C_{11}H_{14}O_3$ | Zingerone | 10 | 974 |
| $C_{15}H_{24}O$ | Santalol | 4.2 | 899 |
| $C_{15}H_{24}$ | 1,6,10-Dodecatriene,7,11-dimethyl-3-Methylene-, (6E)- | 4.1 | 887 |
| $C_{10}H_{18}O$ | Geraniol | 1.4 | 1136 |
| $C_{10}H_{16}$ | Dipentene | 1.4 | 788 |

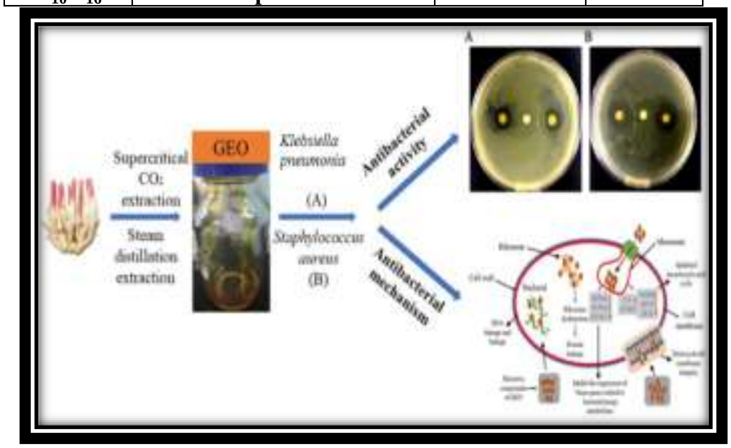


**Figure 2.** Antibacterial activity and mechanism of ginger essential oil against *Klebsiella pneumonia* and *Staphylococcus aureus* [26].

**Isolation and diagnosis of the types of pathogenic bacteria under study:**

Disease-causing bacteria are diagnosed and isolated in many ways, depending on the type of bacteria. In the first stage, bacterial colonies were diagnosed based on their morphological characteristics on the culture media, which include the size, color, edges, and height of the bacterial colonies, their ability to ferment the sugar lactose, their ability to produce mucus, the production of hemolysin, and the formation of the cell membrane. Then the second stage was observing the characteristics of the cells under the microscope after staining with the Gram stain. Different culture media were used to diagnose the isolated bacteria, some of which were differential or selective, such as MacConkey's medium, Mueller-Hinton's medium, eosin agar, blood culture, Mintol salt agar. The isolates were further diagnosed by biochemical tests with three main tests, which are the production of the Catalase enzyme. This test is used to distinguish between *Staphylococcus aureus* (positive) that the blood clotting enzyme is produced by (negative) staphylococci and other types of bacteria. Oxidase fermentation test, as all Gram-negative bacteria ferment this enzyme, while positive ones cannot ferment this enzyme. The third test is the Coagulase test. All types of gram-negative bacteria are negative for this. Testing: Bacteria positive for Gram stain are positive for this test, while partial diagnosis is made with PCR tests and using VITEK2 -API20 technology [27, 28].

**Pathogenicity of antibiotic-resistant Gram-negative and Gram-positive bacteria:**

It has been shown that antibiotic resistance by Gram-negative and Gram-positive bacteria results from certain factors and mechanisms possessed by these dangerous types of bacteria that enable them to resist multiple drugs (MDA) [29]. Among these factors are that these isolates of bacteria contain the ability to form a cell membrane and the ability to produce hemolysin. Likewise, the biofilm on blood agar plates, 29% of the bacterial isolates showed the production of highly toxic compounds, as well as the formation of adhesion proteins, in addition to toxicity resulting from the virulence genes of four types of bacteria, namely *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus*). All the types of bacteria mentioned previously are considered to be producers of biofilms, as was shown in the Congo Red plates test. They were dark red or black and reddish in color (32%). They are undoubtedly producers of biofilms, in addition to the formation of a number of virulence genes, especially in staphylococcal bacteria, and among these are Genes, for example, *finbA*, *clfA*, and *efb*, have appeared as antibiotic resistance genes and were prevalent in most of the isolates under most studies [30]. Among these genes are *sdrD, icaA, coa, nuc, hlg, hla,* and *finbA,* which appeared in staphylococci, while virulence genes were found in The *Klebsiella pneumoniae* bacteria were Kp16s, kpfima and Khe gene)[31]. As for the *Pseudomonas aeruginosa* bacteria, they possess a wide spectrum of virulence genes, including exoenzyme and exotoxin A. In one study, it was found that approximately (63%) of the isolates In otitis media containing multidrug-resistant *Pseudomonas aeruginosa* (MDR-*Pseudomonas aeruginosa*), two isolates (9.5%) were resistant to all eight classes of antibiotics used in the study and were considered PDR, and three bacterial isolates (14.2%) could be resistant to seven classes. of antibiotics, which were considered XDR [32] (Table 2).

The virulence factors possessed by *Bacillus cereus* were detected. The results showed that the two isolates were beta-hemolytic and starch degraders. The samples isolated from food showed their ability to produce the enzyme lecithinase, while the bacteria isolated from the soil did not produce this enzyme. The two isolates also showed their ability to produce the enzyme lecithinase. Protease enzyme production and growth in NaCl 7.5%. The effect of some antibiotics was also studied, as the results of the study showed that the two isolates were resistant to the antibiotics ampicillin and sensitive to amikacin, chloramphenicol, and erythromycin [14] (Table 3).

**Table 2.** Distribution and percentage of PA for patients with ear and respiratory tract infections caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa* [30; 32].

| Type of pathogenic bacteria | The number of isolates in which the bacteria appeared | percentage |
|---|---|---|
| ***Staphylococcus aureus*** | **21** | **33%** |
| ***Pseudomonas aeruginosa*** | **19** | **29%** |
| ***Bacillus* spp.** | **15** | **23%** |
| ***Staohylococuus epidermedis*** | **5** | **8%** |
| ***Klebsiella pneumonia*** | **10** | **15%** |
| **No growth** | **35** | **100%** |

**Table 3.** Antibiotic sensitivity of gram positive bacteria *B. cereus* and *S. aureus* bacteria.

| Antibiotics | Isolation of S. aureus from soil | Isolation of S. aureus from food | Isolation of B.cereus from soil | Isolation of B.cereus from food. |
|---|---|---|---|---|
| | | | | |
| **Ampicillin** | **R** | **R** | **R** | **R** |
| **Amikacin** | **S** | **R** | **S** | **S** |
| **Chloramphenicol** | **R** | **R** | **S** | **S** |
| **Erythromycin** | **R** | **R** | **S** | **S** |
| **Symbols: R Resistance, S Sensitive.** | | | | |

**Extracting active compounds from ginger and thyme:**

The process of extracting the active compounds found in the plants under study is done using various organic solvents, including water, alcohol, or ethanol in certain proportions. These solvents are highly polar and have the ability to extract and extract most of the active substances in plants, such as ginger and thyme. However, there are medium or few organic solvents. Polarity can be used to extract active substances from plants, such as chloroform and hexane, as hexane extracts oily or fatty compounds that are particularly low in polarity. Therefore, it is possible to extract volatile oils from aromatic plants at a high degree of purity using only hexane as a low-polar organic solvent. Low-polarity solvents, however, extract fewer phytochemicals than high-polarity solvents, resulting in lower extract yields. Water extracts large amounts of a variety of tannins, flavonoids, and other polyphenols, while methanol also extracts many of the same compounds. , as well as other polar phytochemicals [33].

**Active substances in ginger and thyme against antibiotic-resistant bacteria:**

**Ginger plant:** It has been proven that the ginger plant contains a mixture of effective compounds that work to stimulate the secretion and activity of digestive enzymes and inhibit the activity of pathogenic bacteria. The most important of these compounds are Shogaols, gingerol, gingerdiol, and ginger) [34] and thus these work. Compounds those are effective in resisting gram-negative and positive pathogenic bacteria. In addition, it was found that this plant leads to an increase in the effectiveness of the immune system by increasing the activity of forming all types of white blood cells and preventing free radicals (Figure 3), in addition to the formation of volatile oils such as flavonoids and terpenes, in addition to esters and some natural organic acids Which has the ability to inhibit and kill antibiotic-resistant bacteria [21, 25].

It has also been shown from the phytochemical composition that camphene and gingerine are the main components of ginger oil, while thymol and eugenol are the main volatiles in thyme and cloves, respectively. It was also shown that the antimicrobial activity of EOS (chemical compounds extracted from ginger and thyme) specifically against seven strains of bacteria causing human diseases, spoilage, yeast and mold at an MIC of 0.001%. It was also shown that the viability of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* in fortified cheese mold showed a complete reduction in *S.* aureus bacteria at the end of the first and second weeks of treatment with thyme and ginger for the infected samples, respectively, and an approximately 50% reduction in *P. aeruginosa* by the end In the fourth week. By conducting tests on samples infected and treated with ginger and thyme extracts only [35].

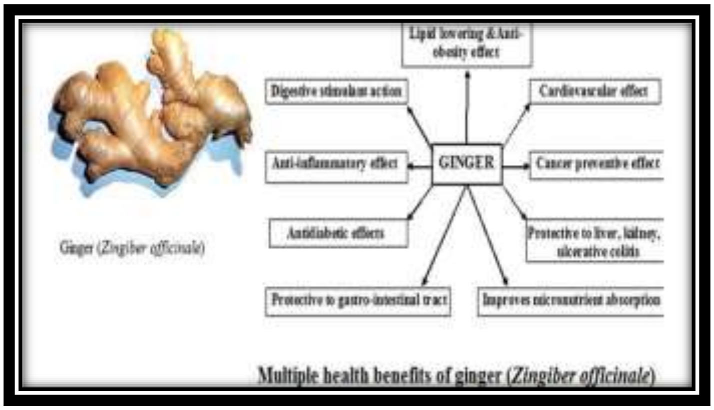


**Figure 3.** Anti-inflammatory and antioxidant activities of ginger.

**Thyme plant**: It has been shown that the effect of the aqueous extract of *Thymus vulgaris* on the bacteria *Klebsiella pneumoniae* and *Staphylococcus aureus* bacteria was carried out. A test was conducted for the sensitivity of bacteria to these extracts and compared to the effect of some antibiotics. The sensitivity test for *K. pneumoniae* bacteria to antibiotics showed that the antibiotic Ceftriaxone (CRO 30µg) was the strongest among the antibiotics used with an inhibition zone of 27 mm, while these bacteria were not affected by the antibiotic Ampicillin (AM). While the results of the sensitivity test for *Staphylococcus aureus* bacteria showed For antibiotics, the antibiotic (SXT 25µg) Trimethoprim /Sulfamethoxazole) was the strongest among the antibiotics used with an inhibition zone of 26mm, while the bacteria were not affected by the antibiotic (AM). The results also showed that the aqueous solution of thyme at a concentration of 210 and 300 mg/ ml showed a clear effect against the bacteria *Klebsiella pneumoniae* with a diameter of 14 mm, while the aqueous solution of *Thymus vulgaris* against the bacteria *S. aureus* showed an inhibition zone with a diameter of 25.5 mm at a concentration of 300 mg/ml and an inhibition zone with a diameter of 20.5 mm at a concentration of 210 mg/ml, respectively. This indicates that there is a relationship. There is a direct correlation between the concentration of the aqueous solution of thyme and the diameter of the inhibition zone in general. The greater the concentration of the aqueous solution of thyme, the greater the diameter of the inhibition zone in particular [35] because of present many active compounds (Figure 4).

**Table 4.** Chemical components of the volatile oils in the thyme plant [33].

| α-Terpinene | β-Myrcene | Linalol | γ-Terpinene | p-Cymene | Carvacrol | Thymol |
|---|---|---|---|---|---|---|
| 1.3 % | 1.5% | 4% | 8% | 20% | 3.5% | 45% |

## Discussion

The results of this systematic study have shown that antibiotic-resistant bacteria, or so-called (MDA), have become resistant to multiple drugs because they possess mechanisms and characteristics through which they can survive despite their exposure to antibiotics. These bacteria are primarily characterized by the properties of the plasma membrane of the bacterial cell. Most types of resistant bacteria prevent the antibiotic is unable to reach the bacterial cell due to the inability of the antibiotic to destroy or penetrate the plasma membrane of the bacterial cell in general. This may occur as a result of hereditary genetic changes resulting from mutations that lead to a change in the nature of the membrane. This change occurs continuously, which always leads to failure. After using the antibiotic successfully for a certain period of time against antibiotic-resistant bacteria [36], this is on the one hand, and on the other hand, these bacteria secrete enzymes and form some types of proteins called adhesion proteins through which they can adhere to cells. The host uses certain mechanisms that then destroy part of the host's cell membrane and enter the host's tissues and cells. Then it reproduces and secretes toxins and organic wastes into the host's tissues, which as a result leads to the destruction of the tissues and cells and the occurrence of the host's pathological condition. Studies have shown that these characteristics that possessed by these disease-causing bacteria resulting from the construction of proteins and enzymes with genetic codes found in DNA, these codes are genes called virulence genes, and this code is transmitted by messenger RNA [1,5]. Each type of antibiotic-resistant bacteria possesses a set of virulence genes. These genes differ in their nature and extent of virulence depending on the type and strain of pathogenic bacteria.

The extracts resulting from the plants under study, ginger and thyme, are in fact secondary metabolic compounds that are naturally produced in these plants from metabolic processes and include many compounds such as phenols, terpenes, flavonoid esters, and other compounds, where many compounds of special importance have been identified. Such as phthalene, also known as isocoumarin, is present in aqueous and methanolic extracts and isocoumarins have been shown to have good inhibitory activity against bacteria (*S. aureus*, *S. epidermidis* , *K. pneumonia* , *P. aeruginosa* , and *B. subtilis*). This includes the presumed mechanisms of action of isocoumarins, which lead to the inhibition of the penicillin-binding protein in Helicobacter pylori [37], in addition to the cell membrane interaction in *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *K. pneumoniae* due to their lipophilic nature relative to the membrane and their planar structure, which to. Penetrating the cell wall, these compounds interact with the membrane, causing deformation and destruction of the basic structure of the bacterial membrane, thus allowing the entry of antibiotics into the bacterial cells, causing distortion or distortion of the bacterial DNA. This may be due to their combination with the nitrogenous bases that make up the bacterial DNA.

It has also become clear that extracts of thyme and ginger contain a number of natural acids that have clear activity against pathogenic bacteria that are generally diagnosed by the (GC-MS) test [38], such as citric acid, acetic acid, tannic acid, and gallic acid. Free (uncomplexed) and ellagic acid, in addition to multiple carbohydrate compounds. These acids lead to a change in the pH or hydrogen environment, in addition to the fact that these acids dissolve the phosphorylated fats in the wall of pathogenic bacteria, causing them to be inhibited and killed.

All studies [19, 20, 26, 34, 35] have proven that ginger and thyme extracts have an inhibitory ability against types of bacteria resistant to antibiotics. The strength of inhibition ranges from 5 mm to 20 mm depending on the type of bacteria. When using the extracts together, this leads to an increase in the inhibition rate to 50%, and this may be due to the equivalence of these compounds. Plants dissolve the lipids that make up the membrane and create holes in the cell membrane that lead to the escape of cellular components out of the cell and ultimately the death of the cell.

**Conclusions**

The most antibiotic-resistant bacteria are *S. aureus*, *K.* pneumoniae, and *P. aeruginosa* because they possesses certain genetic characteristics, formed by virulence genes that enable it to resist multiple drugs. However, it is possible to kill and inhibit these bacteria with ginger and thyme extracts, including volatile oils and chemical compounds. It is also possible to synergize with extracts of these plants and traditional antibiotics to increase the activity and efficiency of the antibiotics and killing bacteria more.

**Conflict of Interest**

No conflict of interest.

**References**

[1] Chandra, P., Unnikr ishnan, M.K., Vandana, K.E.;, Mukhopadhyay, C., Dinesh Acharya, U., Surulivel Rajan, M. &Rajesh, V. (2021) Antimicrobial Resistance and the Post Antibiotic Era: Better Late than Never Effort. *Expert. Opin. Drug. Saf*., 20, 1375–1390.

[2] Şachir, E.E., Puşcaşu, C.G., Preprints, A.C.S., Raftu, G., Badea, F.C. & Mociu, M. (2022). Studies Regarding the Antibacterial Effect of Plant Extracts Obtained from *Epilobium parviflorum*. Appl. Sci., 12(5), 2751.

[3] Stuper-Szablewska, K., Szablewski, T., Przybylska-Balcerek, A., Szwajkowska-Michałek, L., Krzyżaniak, M., Świerk, D., Cegielska-Radziejewska, R & Krejpcio, Z. (2023). Antimicrobial Activities Evaluation and Phytochemical Screening of Some Selected Plant Materials Used in Traditional Medicine, Molecules, 28(1), 244.

[4] Mohammad, L.M; Kamil, A.M.; Tawfeeq, R.K. & Ahmed, S.J. (2023). Ameliorating Effects of Herbal Mixture for Dexamethasone Induced Histological Changes in Mice. *International Journal of Veterinary Science*, 12(1), 126-131.

[5] Pancu, D.F., Buzatu, R., Milutinovici, R., Iurciuc, S., Dolghi, A. & Poenaru, M. (2022). Assessment of the Biological Activity of Thyme Essential Oil in the Presence of the Classic Antibiotic Tetracycline. *Curr Health Sci J.*, 48(1), 18-23.

[6] Dawood, H.N., Zwain, L.A. & Alqaraghuli, H.A. (20210. Bacteriological Profile and Antibiogram of Bacteria in Sputum Culture of Iraqi Patients: A retrospective study. *Ibn AL-Haitham Journal For Pure and Applied Sciences* 34(3), 1-9.

[7] Al-Jubury, K.S., Alshareef, D.K.J., Shakir, S.M. & Al-Jubury, M. (2022). Study Of Some Gram-Negative Bacteria From Blood Cultures Among Neonatal Iraqi Patients And Their Antibiotics Resistance Pattern. HIV Nursing, 22(2), 235-238.

[8] Al-Wandawy, A.H., Zwain, L.A. and Khidher, D.M. (2023). Isolation and Diagnosis of Bacteria in Bacteremia Patients and Study Their Resistance to Antibiotics in Kirkuk Hospitals. *Ibn AL-Haitham Journal For Pure and Applied Sciences* 36.3 (2023), 9-20.

[9] Khalif, A.F. (2016). Study of microbiology and bacterial interaction of fungi associated with nasal and ear infections in patients with diabetes and leukemia. Baghdad Science Journal, Master's thesis, University of Baghdad.

[10] Al-Azzawi, M.H. & Alkalifawi, E.J. (2023). "Detection of Bacteria Causing Burn Infection Isolated from Several Hospitals in Baghdad. *Ibn AL-Haitham Journal For Pure and Applied Sciences* 36(3), 1-8.

[11] Idrees, M.M., Saeed, K., Shahid, M.A., Akhtar, M., Qammar, K.,; Hassan, J., Khaliq, T. & Saeed A. (2023). Prevalence of *mecA*- and *mecC*-Associated Methicillin-Resistant *Staphylococcus aureus* in Clinical Specimens, Punjab, Pakistan. *Biomedicines.*, 11(3), 878. doi: 10.3390/biomedicines11030878.

[12] Umayra, A. N., Hassan, J. S. & Abdul-Hadi, H. F. (2023). Isolation and Identification of Gram-Positive and Gram-Negative Bacteria from Different Types of Cheese in Baghdad's Local Markets. *Biomedicine and Chemical Sciences*, 2(3), 186–189.

[13] Al-Wandawy, A.H.S. & Zwain, L.A. (2020). Ability of Staphylococcus spp. Isolated from Meningitis Patients to Biofilm Formation. *Indian Journal of Forensic Medicine & Toxicology*, 14(4), 1927-1931.

[14] Beecher, D.J. & Wong, A.C.L. (2000). Tripartite Haemolysin BL: isolated and characterization of two Distinct homologous sets of component from a single

Bacillus cereus isolate. *Microbiology*, 146: 1371-1380.

[15] Zbun, S.M. (2021). Study of the inhibitory effect of some therapeutic organisms and plant extracts on biofilm formation of intestinal pathogenic Escherichia coli bacteria isolated from children with diarrhea. College of Science for Girls, University of Baghdad, Master's Thesis.

[16] Al-Azzawi, S.N. & Abdullah, R.M. (2023). Detection of antibiotic resistance of the phylogenetic group E among E. coli bacteria isolated from diarrheal cases in children under five years." *Ibn AL-Haitham Journal For Pure and Applied Sciences* 36(3), 42-50.

[17] Ibrahim, S.K. (2020). Study the effect of linum usitatissimum (flax seeds) and eucalptus rostrata (ecalyptus) leaves extracts on pseudomonas aeruginosa growth in vivo and in vitro. *Biochemical & Cellular Archives* 20(2).

[18] Al-Maliki, A.D.M. (2006), Study of the biological effectiveness of protein extracts and essential oils of thyme (*Thymus vulgaris*) seeds against the growth of pathogenic bacteria in the urinary tract. *Journal of Sight Research (Scientific)*, 32(1), 54-61.

[19] Kim, H-S. & Park, H-D. (2013). Ginger Extract Inhibits Biofilm Formation by *Pseudomonas aeruginosa* PA14. PLoS One. 8(9), e76106.

[20] Hussein, F.A. (2011). Inhibitory effectiveness of aqueous ginger extract against pathogenic microorganisms. *Tikrit Journal for Agricultural Sciences*, 11(4), 30-37.

[21] Kaabour, F. (2009). Activités antioxydantes et antibactériennes desextraits aqueux du thé, de l'origan et du gingembre – Etude *in vitro* Magister. Sciences. Biologie. Microbiologie appliquée. MSc. Thesis, Universite Farhat Abbas –Setif, 23.

[22] Abdul-Jalil, T.Z. (2016). Phytochemicals Screening by GC/MS and Determination of Some Flavonol in Cultivated Iraqi Eruca sativa Dried Leaves Extract and its Biological Activity as Antioxidant.

[23] Spadaccino G., Frabboni L., Disciglio G., Mentana A., Nardiello D. & Quinto M. (2021), Essential oil characterization of *Prunus spinosa* L., *Salvia officinalis* L., *Eucalyptus globulus* L., *Melissa officinalis* L. and *Mentha piperita* L. by a volatolomic approach. *J. Pharm. Biomed. Anal*., 202, 114167.

[24] Zhang, C., Xie, Y., Qiu, W. & Mei, J. (2023). Antibacterial and Antibiofilm Efficacy and Mechanism of Ginger (*Zingiber officinale*) Essential Oil against *Shewanella putrefaciens*, *Plants (Basel)*, 12(8), 1720.

[25] Guerrero, A., Guerrero, E., Cartuche, L. & Morocho V. (2022), Chemical Profiling of *Hedyosmum cumbalense* and *Hedyosmum spectabile* (Chloranthaceae) Essential Oils, and Their Antimicrobial, Antioxidant, and Anticholinesterase Properties. *Plants*, 12, 39.

[26] Wang, X., Shen, Y., Thakur, K., Han, J., Zhang, J-G. & Hu, F. (2020). Antibacterial Activity and Mechanism of Ginger Essential Oil against Escherichia coli and *Staphylococcus aureus*. *Molecules*, 25(17), 3955.

[27] Hasanpour, A.H.; Sepidarkish, M., Mollalo, A., Ardekani, A. & Almukhtar,M. (2023). The global prevalence of methicillin-resistant Staphylococcus aureus colonization in residents of elderly care centers: a systematic review and meta-analysis, *Antimicrobial Resistance & Infection Control*, 12(1), 4.

[28] Hamid, H.N., AL-Obaedi, A.I. & AIjubory, I.S. (2021). Phytochemical Study with Evaluation the Antimicrobial Activity of *Cressa cretica* Plant Against Some Gram-Positive and Gram-Negative Bacteria. *Ibn AL-Haitham Journal For Pure and Applied Sciences* 34(3), 17-25.

[29] Hamady, D.R. & Ibrahim, S.K. (2020). The study on ability of *Escherichia coli* isolated from different clinical cases to biofilm formation and detection of csgd gene responsible for produce curli (fimbriae). *Biochemical & Cellular Archives* 20(2).

[30] Azmi, K..l Qrei, W. & Abdeen, Z. (2019). Screening of genes encoding adhesion factors and biofilm production in methicillin resistant strains of *Staphylococcus aureus* isolated from Palestinian patients. *BMC Genomics*, 20, 578.

[31] Shrestha, S.K., Shrestha, J., Mason, C.J., Sornsakrin, S., Dhakhwa, J.R. & Shrestha, B.R. (2023). Etiology of Acute Diarrheal Disease and Antimicrobial Susceptibility Pattern in Children Younger Than 5 Years Old in Nepal. *Am J Trop Med Hyg*. 108(1), 174–180.

[32] Ibrahim, A.H. (2022). Link Between Some Virulnce Factors Genes and Antibacte Rialresistance of *Pseudomonas aeruginosa*. *Iraqi Journal of Agricultural Sciences*, 53(5), 985-993.

[33] Cheesman, M.J., Alcorn, S., Verma, V. & Cock, I.E. (2021), An assessment of the growth inhibition profiles of *Hamamelis virginiana* L. extracts against *Streptococcus* and *Staphylococcus* spp. J. Tradit. Complement. Med., 11, 457–465.

[34] Al-Taie, H.M.S. & Shanoun, A.Q. (2022). Use of Indian ginger tuber powder (Roscoe officinale Zingiber) it affected the biochemical parameters and

intestinal flora of broiler chickens. *Journal of Agricultural Sciences*, 13(3), 2.

[35] Ahmed, L.I., Ibrahim, N., Abdel-Salam, A.B. & Fahim, K.M. (2021). Potential application of ginger, clove and thyme essential oils to improve soft cheese microbial safety and sensory characteristics. *Food Bioscience*, 42, 101177.

[36] Arbab, S., Buriro, R. S., Ullah, H., Bhugio, S.U., Shah, A. H., Kalho, D.H., Memon, M.A., Tunio, S., Vistro, W.A. & Khoso, A.N. (2021). Comparison of antibacterial activity of Ciprofloxacin and Cephalexin against Some common bacterial Species isolates from donkey wounds around the vicinity of Tandojam Sindh Pakistan. *Pure and Applied Biology (PAB)*, 10, 1095–1103.

[37] Shabir, G., Saeed, A. & El-Seedi, H.R. (2021). Natural isocoumarins: Structural styles and biological activities, the revelations carry on. *Phytochemistry*, 181, 112568.

[38] Sharma, A., Biharee, A., Kumar, A. & Jaitak, V. (2020), Antimicrobial terpenoids as a potential substitute in overcoming antimicrobial resistance. *Curr. Drug Targets*., 21, 1476–1494.

# دراسة تأثير المستخلصات النباتية على مسببات الأمراض الموجبة والسالبة لكرام: مراجعة

**الاء سالم سادة الحلفي [*]، ماهر زكي فيصل الشمري ، سعاد خليل ابراهيم**

قسم علوم الحياة ، كلية التربية للعلوم الصرفة (ابن الهيثم)، جامعة بغداد، بغداد، العراق

aalaa.sada2102p@ihcoedu.uobaghdad.edu.iq

**الخلاصة:**

تشكل البكتيريا المقاومة للمضادات الحيوية الموجبة والسالة لكرام (المعروفة باسم MDA) تهديدًا مستمرًا ومتزايدًا لصحة الإنسان في جميع أنحاء العالم، وخاصة في البلدان الفقيرة. الهدف من الدراسة هو إيجاد بدائل للمضادات الحيوية التقليدية أو استخلاص مركبات فعالة تعمل بالتآزر مع المضادات الحيوية أو كبديل لها. أثبتت العديد من الدراسات قدرة المستخلصات النباتية، وخاصة الزيوت الطيارة الموجودة في النباتات العطرية أو الطبية مثل الزنجبيل والزعتر، على تثبيط أو قتل البكتيريا السالبة لكرام مثل: الزائفة الزنجارية والبكتيريا الموجبة لكرام مثل المكورات العنقودية الذهبية المقاومة للمضادات الحيوية. وقد وجد أن هذه المستخلصات تتكون من مركبات كيميائية. تقوم أحماض الكربوهيدرات بتدمير الأغشية البكتيرية وإذابتها وتدمير الخلية المستهدفة. يتم تشخيص المستعمرات البكتيرية بناءً على خصائصها الشكلية، مثل حجم المستعمرات ولونها وحوافها وارتفاعها، وقدرتها على تخمير سكر اللاكتوز، وقدرتها على إنتاج المخاط والهيمولايسين. تشخيص العزلات باستخدام الاختبارات البيوكيميائية بثلاثة اختبارات رئيسية. ويتم التشخيص الجزيئي عن طريق إجراء فحوصات PCR وباستخدام تقنية (VITEK2). تم استخلاص المركبات النشطة من الزنجبيل والزعتر باستخدام مذيبات عضوية قطبية مختلفة، وتبين أن جينات الضراوة، مثل الإنزيمات والسموم والبروتينات اللاصقة وبروتينات سطح الخلية التي تنتجها المكورات العنقودية الذهبية وأنواع أخرى من البكتيريا المسببة للأمراض، هي المسؤولة. للالتهابات التي تتشكل عند الأشخاص المصابين بهذا المرض. يمكن للمركبات النشطة الناتجة عن مستخلصات الزنجبيل والزعتر أن تثبط وتقتل البكتيريا المقاومة للمضادات الحيوية (MDA) من خلال مركباتها الكيميائية والأحماض الكربوهيدراتية التي تشكلها، مما يؤدي إلى تحلل وانحلال غشاء الخلية وموت الخلية البكتيرية. قد تعمل هذه المستخلصات بشكل تآزري مع بعضها البعض أو مع المضادات الحيوية التي تقتل البكتيريا وتثبطها.

الكلمات المفتاحية: *الزنجبيل، مسببات الأمراض الموجبة والسالبة لكرام، البكتريا المقاومة للمضادات الحيوية، الزعتر، جينات الضراوة.*