International Journal of Theoretical and Applied Research (IJTAR)

ISSN: 2812-5878

Homepage: https://ijtar.journals.ekb.eg

Original article

# Antibacterial Effects of Some Medicinal Plants on MDR Bacterial Pathogen Isolates from Different Sources

**Somaya. S A. Ahmed[1], Nagwa M Sidkey [1], Asmaa S M Mansour [2], Amal A I Mekawey [3]**

[1] *Botany and Microbiology Department, Faculty of Science for Girls, Al-Azhar University, Cairo, Egypt*
[2] *Department of Microbiology and Immunology, National Research Centre, Dokki, Cairo, Egypt*
[3] *The Regional Center of Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt*

**ARTICLE INFO**

Received 16/04/2025
Revised 22/05/2025
Accepted 29/05/2025

***Keywords***

*Medicinal plants*
*MIC*
*MDR*
*Antimicrobial activity*
*Cloves (Syzygium aromaticum)*
*Ginger (Zingiber officinale)*

**ABSTRACT**

Medicinal plants yield numerous antibacterial compounds. These plants possess therapeutic properties and are commonly employed to treat various ailments. In this study, four different medicinal plants (*Zingiber officinale, Syzygium aromaticum, Citrus limon, and Allium sativum*), traditionally used in medicine, underwent preliminary screening for antimicrobial potential against five pathogenic microorganisms: *S. aureus*, *Klebsiella pneumoniae*, *Hafnia alvei*, *Serratia rubidaea*, and *E. coli*, which were isolated from patients from different sources. Multi-drug resistant (MDR) Gram-negative pathogenic microorganisms were selected from isolated sputum and blood samples. The extracts of each plant were prepared and tested for their antibacterial potential and minimum inhibitory concentration (MIC) using the agar well diffusion method. The results indicated that all plant extracts in this study exhibited antibacterial activity against the test pathogens. *Syzygium aromaticum* demonstrated excellent and broad-spectrum activity compared to the other four plant extracts. The most significant result was obtained with ethyl acetate clove extract (26 mm), followed by ginger (22 mm). This study emphasizes the significance of these plant extracts in controlling certain pathogenic microorganisms.

**Graphical abstract**

⁎ *Corresponding author*
*E-mail address*: mmmh262016@gmail.com

DOI: **10.21608/IJTAR.2025.375404.1120**

## 1. Introduction

Infectious diseases are a major source of morbidity and mortality worldwide, accounting for about half of all deaths in tropical countries. Despite significant advancements in microbiology and microorganism management, rare outbreaks caused by drug-resistant bacteria and previously unknown disease-causing microbes pose serious threats to public health. These troubling health trends demand a global initiative to develop new measures for preventing and treating infectious diseases [1].

Medicinal plants are the primary strategy for treating bacterial infections. Antibiotic resistance is an essential issue in clinical use, and it can lead to therapeutic failure. Other problems with certain antibiotics include toxicity, high cost, and poor efficacy. These issues need an ongoing quest for novel antibacterials [2].

Antibiotics are widely used to treat bacterial illnesses, but some pathogenic bacteria are increasingly resistant. Antibiotic overuse has hastened the quest for new antibacterial treatments. Plant-based natural compounds make up the majority of antimicrobial medications. Plants have been beneficial to human health since the beginning of civilization, and their ability to produce antimicrobial compounds has always led to the discovery of new natural antibiotic alternatives [3].

Methicillin-resistant *S. aureus* and extended-spectrum β-lactamase (ESBL)-producing *E. coli* and *K. pneumoniae* are becoming increasingly difficult to treat due to their ongoing development of resistance to most currently available antibacterial drugs through mutation or genetic information exchange. The rate at which multidrug-resistant bacteria are emerging and re-emerging has alarmingly increased, leading to significant treatment challenges and remaining a global clinical and public health concern [4].

Although few studies have been conducted on the antibacterial properties of medicinal herbs, their use has gained popularity worldwide in recent years due to increasing resistance among various types of bacteria, a phenomenon later known as antibiotic resistance. This situation has led many people, especially the poor, to turn to herbs as an alternative to medicines [5].

To address the issue of antibiotic resistance, medicinal plants have undergone extensive research as alternative treatments for various ailments [6]. These plants contain a range of bioactive compounds that give them antibacterial properties, which can be harnessed to develop new antibiotics. By 2050, the international trade value of medicinal plants and their products is projected to reach USD 5 trillion. Plants produce various compounds that, while not part of their primary metabolism, assist in surviving environmental challenges [7].

Palombo (2011) reported that since plant-derived medicines are generally considered safer than synthetic alternatives, their use is expected to increase. There is much hope for discovering new bioactive chemicals because just around 1% of the world's 500,000 plant species have had their phytochemicals examined [8]. Medicinal plants are rich in numerous secondary metabolites and phytochemical substances, such as tannins, alkaloids, and flavonoids, which treat various ailments [9]. The plant uses its secondary metabolites, particularly oleoresins and essential oils, as chemical weapons to defend itself from bacterial invasions. As natural products, spices are botanicals that are quite stunning. These fragrant herbs are utilized for many purposes beyond their original usage. *Zingiber officinale* (ginger) and *Syzygium aromaticum* (clove bud) are two spices that have significant medicinal benefits [10].

Clove (*Syzygium aromaticum*) is a plant commonly cultivated in the Spice Islands, Indonesia, Pemba, and Zanzibar; however, it was initially produced in China. Clove is a common name for the blooming bud of a tree in the Myrtaceae family [11]. Its antibacterial properties were demonstrated when essential oil extracts killed many Gram-positive and Gram-negative species. Clove's essential oils contain eugenol, oleic acids, and lipids, contributing to their antibacterial properties [12].

Ginger (*Zingiber officinale*) is a well-known spice and herbal remedy to cure symptoms such as fever, cough, etc., particularly its antibacterial activity, which has been extensively utilized in clinics or as a preservative in the food sector [13]. According to a chemical investigation, ginger comprises over 400 distinct components. Lipids (3–8%), terpenes, phenolic chemicals, and carbohydrates (50–70%) are the main components of ginger rhizomes. Ginger has direct anti-microbial activity and thus can be used to treat bacterial infections [14].

The lemon tree, or Citrus limon, is a native Asian plant that belongs to the *Rutaceae* family. It is cultivated commercially worldwide in warm temperate, tropical, and subtropical regions, including the Mediterranean. The peels of citrus fruits contain flavonoids, glycosides, coumarins, sitosterol, and volatile compounds, which have an antibacterial effect [15].

An ancient plant, garlic (*Allium sativum*) has influenced many cultures and culinary traditions. In China and India, for example, it is used as a spice, flavoring, and medicinal herb. It is well-liked in many different cuisines due to its distinct flavor, aroma, and health advantages [16]. Botanically, *Allium sativum* belongs to the *Lillaceae* family, which includes onions, chives, and shallots [17]. Garlic contains at least thirty-three sulfur compounds, several enzymes, and seventeen amino acids. Garlic's strong smell and many of its therapeutic properties are caused by sulfur molecules [18]. These sulfur compounds give garlic its well-known therapeutic properties, including anti-inflammatory, anti-cancer, anti-diabetic, antibacterial, antioxidant, cardioprotective, immunomodulatory effects, and flavor [19].

This study is a preliminary screening for certain plant extracts for biologically active chemicals that can be used against MDR bacteria.

## 2. Materials and Methods

### 2.1. Bacterial strains used in the present study

The bacterial isolates were kindly obtained by Dr. Asmaa Kamal in the Biotechnology lab of Al-Azhar University (Girls Branch). The bacteria found belong to *Klebsiella pneumonia, Hafnia alvei, Serratia rubidaea, Staphylococcus aureus, and E. coli. They* were cultured on a Nutrient agar medium mixed with blood and then incubated at 37$^0$C for 24 hours. The bacteria were tested for antibiotic resistance to determine the most MDR bacteria.

### 2.2. Preparation of plant extracts

The extraction procedure involved different organic solvents (such as methanol, ethanol, and ethyl acetate) and cold water, demonstrating various polarities to facilitate the extraction of all hydrophilic and lipophilic bioactive compounds. Extracts were prepared by weighing 10 g of dried plant powder in 100 ml of cold water, methanol (85%), ethanol (95%), and ethyl acetate (99%), and then left to soak for 3 days in dark bottles at room temperature. However, lemon peel and garlic (fresh plants) were soaked in both of the aforementioned solvents used with the other herbs for 10 days at 37$^0$C. The extracts were filtered through Whatman filter paper to obtain a clear filtrate. The filtrate was then concentrated using a rotary evaporator to produce the final plant extracts, which were collected in a sterile dark bottle and stored in the fridge until use **[20, 21].**

Then, the agar diffusion method was carried out according to **Erhonyota *et al.* (2023)** and **Scorzoni *et al.* (2016) [22, 23].** A predetermined volume of inoculum(50µl) at a standardized concentration is evenly distributed throughout the gelled agar plate's surface. A sterile cork borer is used to aseptically punch a hole 8mm to 1 cm in diameter. The boring agar well is then filled with a predetermined volume of plant extract (100 µl), the plates are then put in the refrigerator for 2 hours, and the test microorganism is cultured at the ideal temperature and duration. The diffusion of the plant extract on bacterial plates was incubated at 37°C for 24 hours **[24],** and the inhibition zone of all types of bacteria under the effect of the different types of solvent extracts was measured, and the highest effective extract **[25].**

### 2.3. The antibiotic disc susceptibility testing method

The most common technique used to detect the antimicrobial effect of the antibiotic is the disk diffusion technique, known as the Kirby-Bauer technique [26]. The disk technique uses several antibiotic paper disks affixed to Mueller-Hinton agar (MHA) plates infected with the test strains**.** The chemical then diffuses into the agar and inhibits the germination and growth of the test bacteria. The antimicrobial effects of antibiotics are defined by the size of the inhibition zone (clear area) of the microorganism formed around the antibiotic discs used. The diameter of these regions formed on the agar surface can be measured precisely with a ruler to within a millimeter**.**

### 2.4. Minimum inhibitory concentration (MIC) method

The minimum inhibitory concentration (MIC) is the lowest extract concentration that completely inhibits the visible growth of a microorganism, required to inhibit or kill bacteria, and it serves as the reference for antimicrobial susceptibility testing **[27].** The test is typically conducted by inoculating a bacterial culture into the wells of a plate and applying dilutions across the rows. The MIC is determined by monitoring the minimum concentration necessary to stop bacterial growth [28]. MIC was evaluated only for the ethyl acetate extract of *S. aromaticum* as the most effective clove extract [21].

The minimum inhibitory concentration was measured using the agar well diffusion method. The plates with Mueller-Hinton agar medium were covered with 0.1 mL of bacterial inoculum. A sterile cork-borer cut wells (8 mm in diameter) in agar plates, filling the wells with 1 mL of each plant extract, followed by the 5 microdilutions (0.5, 0.25, 0.125, 0.625, 0.3125). Plates were incubated at 37°C for 24 hours, and the diameter of the inhibitory zone was determined. Antimicrobial agent combinations were tested three times each. Microorganisms with a clean zone of greater than 6mm were judged inhibited [29].

## 3. Results and discussion

### 3.1. Determination of the MDR bacteria

Data in Table 1 showed the effect of different antibiotics against the bacterial strains under study. Where, the antibiotics used were Amoxicillin (AMOX)10mg, Trimethoprim-Sulphamethoxazole (1.25/23.75) mcg (TXS), Cefotaxime30mg (CTX), Gentamycin120mg (CN), Cefoxitin30mcg (FOX), Cefuroxime 30 mg (CXM), Colistin sulphate (CT) 10 mg, Vancomycin 30 mg (VA), Oxacillin 1 (OX), Erythromycin (E15), Streptomycin (S10) and Rifamycin (RD5). R; resistant, S+; low sensitivity.

**Table (1). The effect of different antibiotics against the bacterial strains under study.**

| Antibiotics / Bacterial Strains | AMX | TXS | CTX | CN | FOX | CXM | CT | VA | OX | E15 | Rd5 | S10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Klebsiella pneumonia* | R | R | R | R | R | R | R | R | R | R | S+ | R |
| *Serratia rubidaea* | R | R | R | S+ | R | R | R | S+ | R | R | S+ | R |
| *Hafnia alvei* | R | R | R | R | R | R | R | S+ | R | R | S+ | R |
| *Staphylococcus aureus* | R | R | R | S+ | R | R | R | S+ | R | S+ | S+ | S+ |
| *E. coli* | R | R | R | S+ | R | R | R | S+ | R | S+ | S+ | S+ |

The data in Table 1 showed that all strains resist most antibiotics. ***Klebsiella pneumonia*** represents the highest strain in antibiotic resistance because it is resistant to all antibiotics used, except for one type (Rifamycin RD5), which has a weak response. It is followed by *Hafnia alvei*, *Serratia*, *Rubbidea*, *Staphylococcus aureus,* and *E. coli.* These results indicate that all strains are MDR.

The present study mainly focuses on determining activity against multidrug-resistant bacteria isolated from the respiratory tract.

### 3.2. Antimicrobial activity of the alcoholic and aqueous plant extracts against the MDR isolates

The results presented in Table 2 indicate that *Syzygium aromaticum* exhibits the strongest effect on *Klebsiella pneumonia*, with inhibition zones measuring 26.00 mm for ethyl acetate, 22.00 mm for ethanol, 19.00 mm for methanol, and 15.00 mm for water. *Zingiber officinale* ranks second, demonstrating inhibition zones of 22 mm for ethyl acetate, 16.33 mm for ethanol, 16.00 mm for methanol, and 14.00 mm for water. Both *Allium sativum* and *Citrus limon* show the least effect overall, although ethyl acetate produced favorable results for them as well. These findings clearly suggest that ethyl acetate extracts yield the most promising results against all multidrug-resistant (MDR) bacteria, followed by ethanol, methanol, and water, which generally exhibit the least impact among the tested extracts.

Many authors demonstrated that plants are known for their essential oils and phenolic chemicals, which prevent oxidative processes and the proliferation of various [28, 30, 31]. Antibiotic-resistant bacteria, such as *K. pneumoniae*, have virulence genes that allow them to resist many medicines. Ginger extracts containing volatile oils and chemical components can effectively kill, eliminate, and suppress bacteria [32]. Ethanol extracts of garlic and ginger have antibacterial action against *Klebsiella spp*. multi-drug resistant isolates [33].

The Clove essential oil (EO) extract displayed the highest activity levels against *K. pneumoniae.* Methanol extracts from several plants were potent against all pathogenic bacterial strains tested, but their inhibition zones differed. The most significant effect is probably with *Klebsiella pneumoniae* [34].

One hundred two multidrug-resistant *Klebsiella* species produce Extended-Spectrum β-lactamase (ESBL) enzymes, which are associated with severe morbidity and mortality and have been observed more frequently in critical care units. Forty-four isolates produced high and moderate levels of biofilm, while 52 isolates were ESBL, indicating that ginger, clove, and garlic oils inhibited biofilm production [35].

Salisu *et al.* reported that the clove extracts contained alkaloids, glycosides, saponins, tannins, flavonoids, sterols, and triterpenes. The antibacterial efficacy of diethyl ether, ethanolic, and aqueous clove extracts was evaluated against multidrug-resistant isolates. *S. aureus* and *Klebsiella* spp. are the most susceptible to all clove extracts, whereas *E. coli* is less so [36].

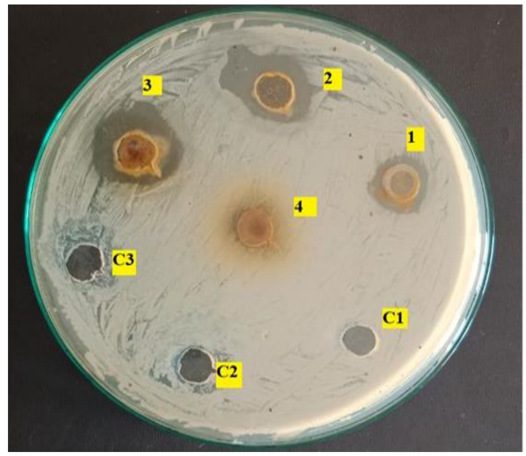


**Figure 1.** The inhibition zone of *Syzygium aromaticum* extracts against *Klebsiella pneumonia* (1; methanol extract, 2; ethanol extract, 3; ethyl acetate extract,4; water extract c1; control methanol, c2; control ethanol, c3; control ethyl acetate)

The EOs with the highest antimicrobial activity levels were cloves *(Syzygium aromaticum L.).* Combined disk diffusion tests and checkerboard assays revealed synergy between these EOs and colistin. Clove extracts are reported to demonstrate significantly high free radical scavenging and peroxide inhibition activity, indicating their reducing character, which may somewhat explain the inhibition of bacterial growth**.** Clove essential oil (CEO) is extracted from cloves, and its chemical composition varies with the growth environment of cloves. In general, eugenol is the main component of CEO, followed by eugenol acetate and caryophyllene**[37].** Figure 1: indicates that the ethyl acetate extract of ***Syzygium aromaticum*** has the highest antimicrobial activity.

**Table (2). Effect of different solvent plant extracts on *Klebsiella pneumonia*.**

| Plant extracts | $H_2O$ extract | Methanol extract | Ethanol extract | Ethyl-acetate extract |
|---|---|---|---|---|
| | The mean diameter of the inhibition zone (mm.) | | | |
| *Zingiber officinale* | 14.00 ± 00.00 | 16.00 ± 00.00 | 16.33 ±0.577 | 22.00 ±00.00 |
| *Syzygium aromaticum* | 15.00 ± 00.00 | 19.00 ± 00.00 | 22.00 ± 00.00 | 26.00 ± 00.00 |
| *Citrus limon* | 00.00 ± 00.00 | 15.00 ± 00.00 | 16.00± 00.00 | 19.00 ± 00.00 |
| *Allium sativum* | 00.00 ± 00.00 | 15.00 ± 00.00 | 16.00 ± 00.00 | 20.00 ± 00.00 |

Clove extracts reportedly possess antioxidant and antimicrobial properties due to the presence of phenolic compounds, such as flavonoids and hydroxybenzoic acids. Ethyl acetate was the most effective organic solvent in the extraction process, producing a high yield of clove-active constituents [21].

The results shown in Table 3 indicate that *Syzygium aromaticum* extracts have the highest effect on *Staphylococcus aureus,* followed by *Zingiber officinale. Allium sativum* and *Citrus limon* have similar results in all extracts, with moderate results compared to cloves and ginger.

Tshabalala *et al.*(2021) indicated that clove exhibited the greatest inhibition zone extract and outstanding antibacterial activity against the most dangerous pathogens [38].

Gram-positive bacteria were found to be more susceptible to antimicrobial substances in spices than Gram-negative bacteria. The degree of sensitivity varied with the tension and environmental conditions applied. Certain spices can directly influence the fermentation rate by boosting acid production in the starting cultures. Phenols, alcohols, aldehydes, ketones, ethers, and hydrocarbons have been identified as key antibacterial components in spices. The antibacterial activity and mechanisms of action of spices, and their key antimicrobial components, are discussed [39]. Garlic extracts inhibited more bacterial isolates than ginger extracts. In general, ginger extracts have minimal antibacterial activity. However, some ginger extracts were more effective against certain bacteria. However, the results were mixed due to differences in ginger preparations and strengths. Ginger extracts may not have demonstrated antibacterial action against clinical isolates due to a lack of active components or insufficient quantities [40]. The most effective ethanol extracts against *S. aureus* were those made from clove and garlic spices. *S. aureus* showed the maximum sensitivity in the clove extract condition, followed by *Klebsiella sp* [41].

Ethanol and ethyl acetate extracts showed potent antibacterial properties against *Escherichia coli*, *Serratia marcescens*, and *Staphylococcus aureus* ATCC 6538. Ethanol outperformed water when used as a solvent to extract flavonoids and phenolic components from clove buds [42].

Results illustrated in Table 4 indicated that, *Syzygium aromaticum* is evident has the highest effect compared to the other plant extracts and is followed by *Zingiber officinale* against *Serratia rubbidea.* It is also clear that there is a clear difference, which is that lemon peel has a higher effect this time than garlic**.**

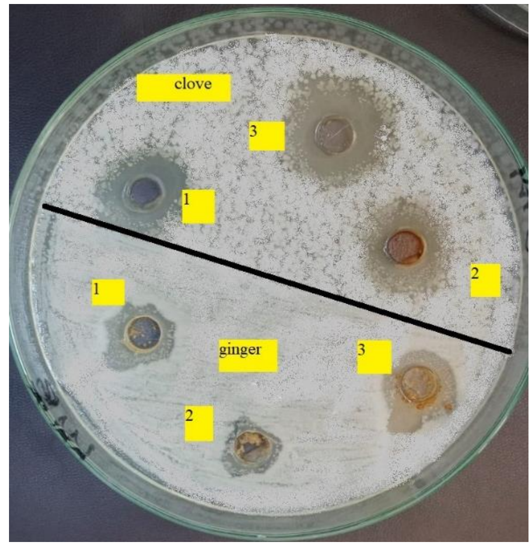


**Figure 2.** The difference in inhibition zones between *Syzygium aromaticum* extracts and *Zingiber officinale* against *Staphylococcus aureus* (1; methanol extract, 2; ethanol extract, 3; ethyl acetate extract, c1; control methanol, c2; control ethanol, c3; control ethyl acetate ). The antimicrobial activity of clove is higher than that of ginger

Plant extracts have a very complex structure, with the active components taking the form of natural organic molecules. The procedure of extracting a specific molecule is determined by its solubility in the solvent (aqueous or organic). The technique and extraction system vary with each product and chemical. The crude extracts of the studied plants revealed promising antibacterial activity [43].

**Table (3):** Effect of different solvent plant extracts on *Staphylococcus aureus*

| Plant extracts | $H_2O$ extract | | Methanol extract | Ethanol extract | Ethyl-acetate extract |
|---|---|---|---|---|---|
| | | **The mean diameter of the inhibition zone (mm.)** | | | |
| ***Zingiber officinale*** | 20.00 ± 01.00 | | 19.00 ± 01.00 | 21.00 ± 1.00 | 22.00 ± 1.00 |
| ***Syzygium aromaticum*** | **18.00 ± 00.00** | | **19.00 ± 00.00** | **23.00 ±2.00** | **25.00 ± 1.732** |
| ***Citrus limon*** | 00.00 ± 00.00 | | 12.00 ± 00.00 | 13.00 ± 1.00 | 14.00 ±00.00 |
| ***Allium sativum*** | 00.00 ± 00.00 | | 12.00 ± 00.00 | 12.00 ± 00.00 | 13.00 ±1.00 |

**Table (4):** Effect of different plant extracts on *Serratia rubidaea*

| Plant extracts | $H_2O$ extract | Methanol extract | Ethanol extract | Ethyl-acetate extract |
|---|---|---|---|---|
| | **The mean diameter of the inhibition zone (mm.)** | | | |
| ***Zingiber officinale*** | 14.00 ± 00.00 | 16.00 ±00.00 | 16.33 ±1.527 | 22.66±1.154 |
| ***Syzygium aromaticum*** | **18.00 ± 00.00** | **19.66 ± 00.50** | **22.00± 00.00** | **26.00±01.00** |
| ***Citrus limon*** | 00.00± 00.00 | 11.00 ±01.00 | 11.00 ± 01.00 | 12.00 ± 00.00 |
| ***Allium sativum*** | 00.00 ± 00.00 | 15.00± 01.00 | 15.00 ± 01.00 | 21.00 ± 00.00 |

Spices may have considerable promise as antibacterial agents. Some bacteria resistant to certain antibiotics were susceptible to extracts of both garlic and clove [44].

As many previous studies have shown that, the extract of clove EO (essential oil) demonstrated the highest antimicrobial activity and antimicrobial spectra against Gram-positive bacterial strains and Gram-negative bacteria, indicating that the essential oil, which is thought to make up the majority of the plant's composition, contains flavonoids, which are active substances that affect bacteria [45].

*Syzygium aromaticum* ethyl acetate extract showed the highest antimicrobial zone against *Serratia*. Clove extract was the most inhibitory, followed by extracts of ginger and garlic, which showed weak antibacterial activities against the tested strains [46]. Ethanol extracts of garlic and clove showed broad-spectrum antibacterial activity against *Serratia sp* [47].

The US FDA has designated clove oil, a pale yellow liquid derived from the leaves, buds, and stems of *Syzygium aromaticum*, as a GRAS (Generally Recognized As Safe) component for human consumption. It's been a food ingredient for many years [48].

According to the findings, there were 65.52 mg of total phenolic compounds per gram of extract (GAE/g) in the ethyl acetate fraction of clove leaves. Therefore, it is possible to develop the ethyl acetate fraction of clove leaves as a source of phenolic compounds [49].

Garlic and ginger are natural spices with strong antibacterial properties against clinical infections resistant to several drugs. They can also be utilized to prevent drug-resistant microbial illnesses [50].

The data recorded in the provided table (Table 5) shows a different result from the previous one. It proves that *Zingiber officinale* has the highest effect compared to the other plant extracts, and is followed by *Syzygium aromaticum* against *Hafnia alvei*. *Allium sativum and Citrus limon* have similar results, but less than cloves and ginger.

According to the present study, which showed that the antibacterial and therapeutic qualities of ginger extracts, all extracts except water exhibit antibacterial activity, and the suppression of bacterial growth is dose-dependent. Extracts from ginger may be able to cure bacterial illnesses [51].

This study discussed plants including *Zingiber officinale* (ginger) roots, *Citrus limon* (lemon) leaves, and *Syzygium aromaticum* (clove). These plants clearly affect *Hafnia alvei*, which is regarded as one of the examined species [52].

Ottou *et al.* (2022) reported that it can explain why *Syzygium aromaticum* and *Zingiber officinale* do so well in treating specific disorders. It has effectively treated infantile diarrhea, pneumonia, cough, and venereal infections. Furthermore, tannins' capacity to prevent and repair damage produced by reactive oxygen species (ROS) makes them essential for stimulating the immune system and lowering the risk of cancer and other degenerative disorders [28].

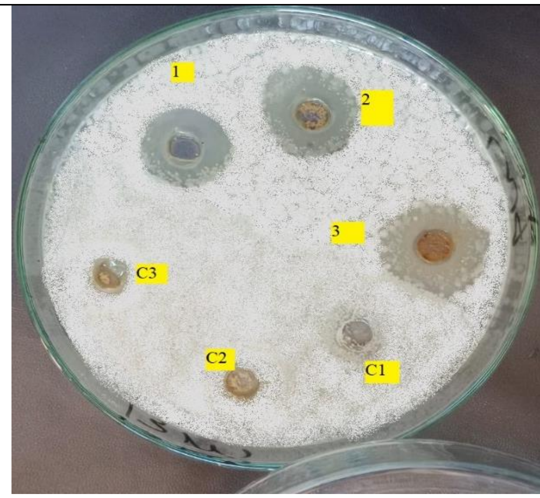


**Figure (3).** The inhibition zone of *Syzygium aromaticum* solvent extracts against *Serratia rubidaea*. (1; methanol extract, 2; ethanol extract, 3; ethyl acetate extract, c1; control methanol, c2; control ethanol, c3; control ethyl acetate )

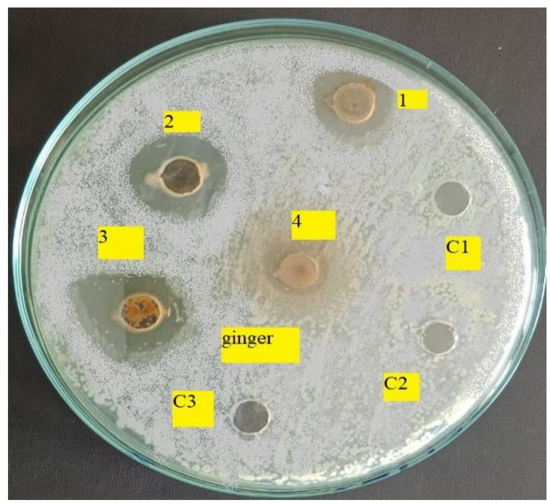


**Figure 4.** Inhibition zones of *Zingiber officinale* extract against *Hafnia alvei*. (1; methanol extract, 2; ethanol extract, 3; ethyl acetate extract,4; water extract, c1; control methanol, c2; control ethanol, c3; control ethyl acetate).

The results illustrated in Table 5 indicated that ethyl acetate extract of *Syzygium aromaticum* has the highest effect against *Escherichia coli*, followed by methanol and ethanol extracts. In addition, the water extract and the ethanol extract of *Zingiber officinale* also gave nearly the same results.

During the pandemic, a mixture of garlic (*Allium sativum*), ginger (*Zingiber officinale*), and clove (*Syzygium aromaticum*) infusion with lemon (*Citrus limon*) was popular. Secondary metabolites such as Allicin from *Allium sativum*, Gingerol from *Zingiber officinale*, Hesperidin, Naringenin, and Quercetin from *Citrus limon* have shown significant benefits against COVID-19 disease. These compounds provide plants with antiviral, antibacterial, antiparasitic, antidiarrheal, anti-inflammatory, antioxidant, and analgesic characteristics, aiding in treating respiratory illnesses [16].

The antimicrobial activity of S. aromaticum is utilized in the treatment of infections by practitioners of traditional medicine. The antimicrobial activity of aqueous extracts, hydroethanolic extracts (which were highly active against all tested germs), and ethanolic extracts may be related to specific phytochemical compounds found in the plant. The toxicity of the extracts must be addressed to develop traditionally improved medicinal products that can replace alcoholic herbal infusions of medicinal plants, as alcohol poses toxicity risks to the organism [53].

The essential oil of *S. aromaticum* had an outstanding antibacterial effect against pathogenic bacteria. The antibacterial mechanism of *S. aromaticum* essential oil on the most resistant pathogenic isolates was more effective against Gram-positive bacteria, requiring lower concentrations to inhibit or kill *S. aureus* compared to Gram-negative bacteria [54].

**Table (5):** Effect of different plant solvent extracts on *Hafnia alvei*

| Plant extracts | $H_2O$ extract | Methanol extract | Ethanol extract | Ethyl-acetate extract |
|---|---|---|---|---|
| | **The mean diameter of the inhibition zone (mm.)** | | | |
| ***Zingiber officinale*** | **16.00 ± 01.00** | **18.00 ± 00.00** | **18.66 ±1.527** | **20.00 ± 00.00** |
| ***Syzygium aromaticum*** | 14.00 ± 00.00 | 15.00 ± 01.00 | 18.00 ± 00.00 | 20.00 ±01.00 |
| ***Citrus limon*** | 00.00 ± 00.00 | 15.00 ± 00.00 | 16.00 ± 00.00 | 20.00 ± 00.00 |
| ***Allium sativum*** | 00.00 ± 00.00 | 16.00 ± 00.00 | 16..00 ± 00.00 | 19.00 ± 00.00 |

**Table (6):** Effect of different plant solvent extracts on *Escherichia coli.*

| Plant extracts | $H_2O$ extract | Methanol extract | Ethanol extract | Ethyl-acetate extract |
|---|---|---|---|---|
| | **The mean diameter of the inhibition zone (mm.)** | | | |
| ***Zingiber officinale*** | 17.00±00.00 | 18.00±00.00 | **22.00 ± 00.00** | 23.33 ± 1.527 |
| ***Syzygium aromaticum*** | **18.00±00.00** | **20.33 ± 0.577** | 21.66 ± 0.577 | **23.66±1.527** |
| ***Citrus limon*** | 00.00±00.00 | 15.00 ± 00.00 | 16.00 ± 01.00 | 20.00 ± 01.00 |
| ***Allium sativum*** | 00.00±00.00 | 16.00±00.00 | 16.00 ± 00.00 | 19.00 ± 00.00 |

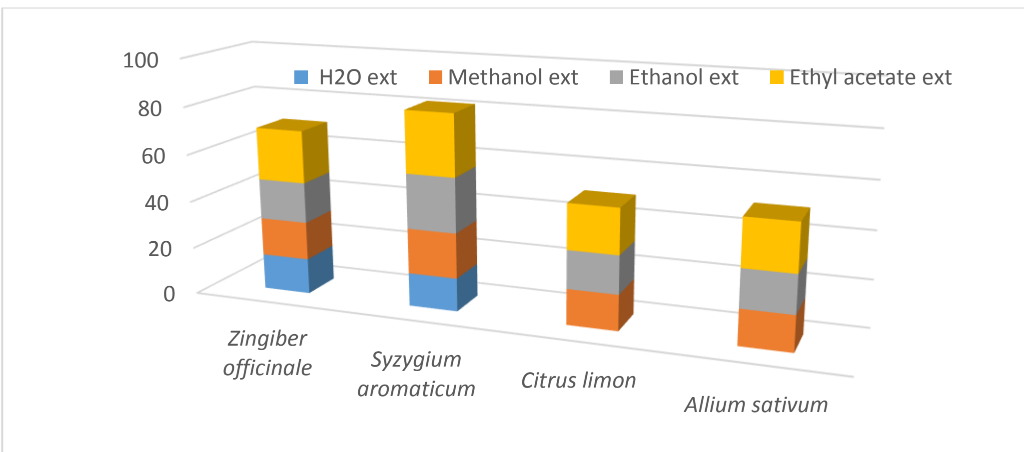


**Figure 5.** Comparison between the plant solvent extracts according to their antibacterial activity.

The antibacterial activity of *Syzygium aromaticum* extracts was the most effective against the MRD isolates, according to the data in tables 2, 3, 4, 5, and 6, followed by the other plant extracts in the following order: *Zingiber officinal*> *Citrus limon* > *Allium sativum* with ethyl acetate as the highest extract in the inhibition zone (mm.).

The results demonstrated that the *Syzygium aromaticum* ethanol extract had considerably more antibacterial activity than the aqueous extract against all tested organisms of *Klebsiella pneumoniae, Escherichia coli,* and *Staphylococcus aureus*. This outcome may be the consequence of ethanol's capacity to extract a greater variety of antibacterial chemicals from clove buds. For both Gram-positive and Gram-negative bacteria, the ethanolic extract consistently generated bigger inhibition zones, indicating a more potent antibacterial activity. This shows that the antibacterial qualities are more closely associated with the kind of extract [55].

*Zingiber officinale* contains resins, terpenoids, flavonoids, alkaloids, and carbohydrates, but lacks tannins, anthraquinones, and saponins. These findings align with the study, which found traces of flavonoids, carbohydrates, tannins, sterols, and terpenoids but no alkaloids [56].

This study found that *Allium sativum* (garlic), *Citrus limon* (lemon), and *Zingiber officinale* (ginger) have stronger antibacterial properties than other antibiotics against *Escherichia coli, Staphylococci aureus,* and

*Klebsiella pneumoniae*. This supports the scientific validity of traditional medicine, the Quran, and hadiths for disease treatment [57].

**4. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**

Data in Table (7) indicate the MIC of *Syzygium aromaticum ethanol extract* against *S. aureus, Hafnia alvei,* and *E. coli is 0.625 mg/ml*, but against *Serratia rubbidea and Klebsiella. pneumonia is 0.125mg/ml.*

It was found that clove essential oil (CEO) showed an inhibition effect on *S. aureus* with the MIC of 0.52 mg/mL and could remove 40 % of its biofilm at this concentration. However, the inhibitory mechanism of CEO on *S. aureus* biofilm, especially the molecular mechanism, is still unclear [58].

Similarly, Bai *et al.* reported that CEO's MIC values against *E. coli* were 0.64 and 1.28 mg/mL, respectively, higher than those against *S. aureus* (0.52 and 1.04 mg/mL, respectively). The single peptidoglycan layer that makes up the cell wall of Gram-positive bacteria has limited capacity to stop the entry of antibacterial agents. However, Gram-negative bacteria have a very complicated cell wall construction that limits the passage of foreign, damaging chemicals into their cells. This structure includes peptidoglycan layers, lipopolysaccharides on the outside membrane, and outer membrane proteins [59].

Moreover, plant extract inhibits *E coli* and *S. aureus* activity by 50 % when 90, 85, 64, 120, 80, 124, and 83 μg/mL of plant extract is added to each of the strains [60].

Data in Table 8 indicate the MIC of *Zingiber officinale* ethyl-acetate extract against *Klebsiella pneumoniae, Hafnia alvei, Serratia rubbidea*, and *E. coli. were 0.125* and *S. aureus 0.625*

When Z. officinale is extracted in its organic or crude form, it is a potent antibacterial agent. Ginger's antimicrobial action results from modifications to the cell wall's permeability. Another significant aspect of *Z. officinale's* anti-carcinogenicity is its suppression of glucans or biofilms. The efficacy of *Z. officinale* against cariogenic bacteria is determined by the MIC and MBC, and it varies based on microbiological and analytical parameters [61].

Using agar-disk diffusion and minimum inhibitory concentration, the antibacterial and antifungal properties of essential oils and oleoresins isolated from *Zingiber officinale* (ginger) and *Syzygium aromaticum* (clove bud) are examined in vitro **[62].**

**Table (7):** Minimum inhibitory concentration (MIC) of *Syzygium aromaticum* ethyl-acetate extract.

| concentration (mg/ml) | **The mean diameter of the inhibition zone of *Syzygium aromaticum* (mm.)ethyl-acetate extract.** | | | | |
|---|---|---|---|---|---|
| | Bacterial strains | | | | |
| | *S. aureus* | *K. pneumonia* | *H. alvei* | *S. rubbidea* | *E. coli* |
| 1 | 29.33±1.154 | 21.66±0.577 | 26.33±1.527 | 32.00±01.00 | 31.33±1.527 |
| 0.5 | 25.00±1.00 | 18.33±0.577 | 24.66±0.577 | 24.00±01.00 | 25.33±0.577 |
| 0.25 | 17.33±0.577 | 14.66±0.577 | 21.00±01.00 | 20.33±0.577 | 18.33±0.577 |
| 0.125 | 15.00±00.00 | 11.66±0.577 | 16.33±0.577 | 17.66±1.154 | 14.66±0.577 |
| 0.625 | 12.00±01.00 | 00.00±0.100 | 14.66±0.577 | 00.00±00.00 | 11.66±0.577 |
| 0.3125 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |

**Table (8):** Minimum inhibitory concentration (MIC) of *Zingiber officinale* ethyl-acetate extract.

| concentration (mg/ml) | **The mean diameter of the inhibition zone of *Zingiber officinale* (mm.)ethyl-acetate extract.** | | | | |
|---|---|---|---|---|---|
| | Bacterial strains | | | | |
| | *S. aureus* | *K. pneumonia* | *H. alvei* | *S. rubidaea* | *E. coli* |
| 1 | 25.00±00.00 | 26.66±0.577 | 26.33±1.527 | 30.00±01.00 | 26.66±1.527 |
| 0.5 | 20.33±1.527 | 20.33±0.577 | 24.66±0.577 | 24.00±01.00 | 18.33±0.577 |
| 0.25 | 17.33±0.577 | 15.00±00.00 | 21.00±01.00 | 20.33±0.577 | 14.66±0.577 |
| 0.125 | 15.00±00.00 | 11.66±0.577 | 16.33±0.577 | 17.66±1.154 | 11.66±0.577 |
| 0.625 | 12.00±01.00 | 00.00±0.100 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |
| 0.3125 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |

In the present study, starting from 2.5 mg/mL, all extracts effectively prevented *E. coli* from growing. In general, as the concentration of the extract increased, so did the inhibition zones. All of the studied bacteria were inhibited by ginger in both aqueous and organic solvent extracts. In line with other findings, ginger exhibits antibacterial qualities. The phenolic chemicals gingerol and shogaol, which are active components in ginger, may have antimicrobial properties **[63].**

Data in Table 9 indicates that the MIC of *Citrus limon* ethyl-acetate extract against *Klebsiella pneumonia, Hafnia alvei, Serratia rubbidea* is *0.25 mg/ml* and *S. aureus* and *E. coli* is *0.125mg/ml.*

Considering the evident scarcity of safe and effective antibacterial medications, the fact that both *C. limon* extracts demonstrated a wide range of antibacterial activity and were as effective as synthetic antioxidants emphasizes the plant's medicinal value as a possible source for drug development. It also supports the ethnotherapeutic claim of the plant. Given its nutritional richness and demonstrated strong antibacterial and antioxidant qualities, *C. limon* should be encouraged to avoid health issues through diet **[64].**

**Table (9):** Minimum inhibitory concentration (MIC) of *Citrus limon* ethyl-acetate extract.

| concentration (mg/ml) | **The mean diameter of the inhibition zone of *Citrus limon*(mm.) ethyl-acetate extract.** | | | | |
|---|---|---|---|---|---|
| | Bacterial strains | | | | |
| | *S. aureus* | *K. pneumonia* | *H. alvei* | *S. rubbidea* | *E. coli.* |
| 1 | 20.00±00.00 | 18.00±00.00 | 19.00±00.00 | 20.00±00.00 | 21.00±00.00 |
| 0.5 | 17.33±0.577 | 14.66±0.577 | 15.00±00.00 | 17.66±1.154 | 18.33±0.577 |
| 0.25 | 15.00±00.00 | 11.66±0.577 | 11.00±00.00 | 14.66±0.577 | 14.66±0.577 |
| 0.125 | 11.66±0.577 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 11.66±0.577 |
| 0.625 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |
| 0.3125 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |

**Table (10):** Minimum inhibitory concentration (MIC) of *Allium sativum* ethyl-acetate extract.

| concentration (mg/ml) | **The mean diameter of the inhibition zone of *Allium sativum*(mm.) ethyl-acetate extract.** | | | | |
|---|---|---|---|---|---|
| | Bacterial strains | | | | |
| | *S. aureus* | *K. pneumonia* | *H. alvei* | *S. rubbidea* | *E. coli.* |
| 1 | 19.00±00.00 | 18.00±00.00 | 16.33±0.577 | 17.00±00.00 | 20.00±00.00 |
| 0.5 | 17.33±0.577 | 15.00±00.00 | 14.66±0.577 | 13.33±0.577 | 16.00±00.00 |
| 0.25 | 14.66±0.577 | 11.00±00.00 | 00.00±00.00 | 11.00±00.00 | 12.00±00.00 |
| 0.125 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |
| 0.625 | 00.00±00.00 | 00.00±0.100 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |
| 0.3125 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 | 00.00±00.00 |

Data in Table 10 indicated that **(MIC**) of *Allium sativum* ethyl-acetate extract against *S. aureus, Klebsiella. pneumonia, Serratia. rubbidea, E. coli.* at 0.25mg/ml except for *Hafnia alvei* at 0.5mg/ml

The MIC of local garlic for various solvent extracts. The concentration of the various extracts caused a substantial variation in the Diameter of the Inhibition Zone (DIZ). Beginning at 2.5 mg/mL, all extracts prevented *E. Coli* from growing. Extracts of water, acetone, and ethanol were effective in suppressing the growth of *S. aureus* at 2.5 mg/mL and methanol extract at 10 mg/mL, respectively. The inhibition effect of acetone extract (15.33 mm) was significantly higher than other extracts at 2.5 mg/mL against *E. coli* [63].

Garlic extracts demonstrated lower MIC values than ginger extracts when tested against bacterial isolates. When compared to cloves, the MIC of garlic demonstrated low sensitivity against the majority of the investigated bacteria (*E. coli, S. aureus, k. pneumonia)* [40, 47].

## 5. Conclusion

In this study, it has been found that the plant solvent extracts under study have a strong effect on the MDR( multi-drug resistant) pathogenic strains and the highest extract in its inhibition zone is the ethyl-acetate extract of the ***Syzygium aromaticum*(clove),** and the ethyl acetate in general for all plant extracts except for lemon was higher than the others solvent extracts and this indicates the presence of active compounds which have an antibacterial effect against pathogenic bacteria. Both *S. aureus* and *E. coli* produce the best minimum inhibitory concentration **(MIC)** results. Overall, this study proved to be a good alternative in the current era and a successful replacement for pharmaceuticals with natural plant extracts. More studies must be carried out to isolate the most active substances from the active plant solvent extracts.

## List of abbreviations

**MDR** Multidrug-resistant
**COVID-2019** Coronavirus disease 2019
**EO** Essential oil
**MIC:** Minimum inhibitory concentration
**ROS** Reactive Oxygen Species
**USD** United States dollar
**MHA** Mueller Hinton agar
**GRAS** Generally Recognized As Safe
**DIZ** Diameter of the Inhibition Zone

## Acknowledgment

I am very grateful to all my supervisors for this research for getting these wonderful results, and I ask God to crown this work with success.

## Ethics information

Non

## Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors**.**

## References

1. Mahady GB. Medicinal plants for the prevention and treatment of bacterial infections. Current pharmaceutical design. 11(19) (2005 Jul 1)2405-2427. DOI: https://doi.org/10.2174/1381612054367481
2. Ngemenya MN, Mbah JA, Tane P, Titanji VP. Antibacterial effects of some Cameroonian medicinal

plants against common pathogenic bacteria. African Journal of Traditional, Complementary and Alternative Medicines. 3(2) (2006 Jan 12) [cited 2025 Jun. 4];84-93. DOI: https://athmsi.org/journals/index.php/ajtcam/article/view/81
3. Mirzaei B. Investigation of antibacterial effects of medicinal plants on bacterial pathogens of patients. Med biotech Journal.1(2) (2017 Jun 1)81-85. https:// DOI.org/ 10.22034/MBT.2017.60379
4. Gadisa E, Weldearegay G, Desta K, Tsegaye G, Hailu S, Jote K, Takele A. Combined antibacterial effect of essential oils from three most commonly used Ethiopian traditional medicinal plants on multidrug-resistant bacteria. BMC Complementary and Alternative Medicine. 19(2019):1-9. https://doi.org/10.1186/s12906-019-2429-4
5. Voravuthikunchai SP, Limsuwan S. Medicinal plant extracts as anti–Escherichia coli O157: H7 agents and their effects on bacterial cell aggregation. Journal of Food Protection. 69(10) (2006 Oct 1):2336-2341. https://doi.org/10.4315/0362-028X-69.10.2336
6. Chomnawang MT, Surassmo S, Nukoolkarn VS, Gritsanapan W. Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. Journal of ethnopharmacology.101(1-3)(2005);330-333. https://doi.org/10.1016/j.jep.2005.04.038
7. Acharjee M, Zerin N, Ishma T, Mahmud MR. In-vitro anti-bacterial activity of medicinal plants against Urinary Tract Infection (UTI) causing bacteria along with their synergistic effects with commercially available antibiotics. New Microbes and New Infections. 51(2023): 101076. https://doi.org/10.1016/j.nmni.2022.101076
8. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evidence-Based Complementary and Alternative Medicine. 1(2011);680354. https://doi.org/10.1093/ecam/nep067
9. Stratev D, Zhelyazkov G, Noundou XS, Krause RW. Beneficial effects of medicinal plants in fish diseases. Aquaculture International. 26 (2018) 289–308. https://doi.org/10.1007/s10499-017-0219-x
10. Sethunga M, Ranasinghe MM, Ranaweera KK, Munaweera I, Gunathilake KD. Synergistic antimicrobial activity of essential oils and oleoresins of cinnamon (Cinnamomum zeylanicum), clove bud (Syzygium aromaticum) and ginger (Zingiber officinale). Biocatalysis and Agricultural Biotechnology. 51(2023). 102800.https://doi.org/10.1016/j.bcab.2023.102800
11. Nirmala MJ, Shiny PJ, Raj US, Saikrishna N, Nagarajan R. Toxicity of clove (Syzygium aromaticum) extract. In Clove (Syzygium Aromaticum). (2022);663-674. https://doi.org/10.1016/B978-0-323-85177-0.00007-0
12. Nzeako BC, Al-Kharousi ZS, Al-Mahrooqui Z. Antimicrobial activities of clove and thyme extracts. Sultan Qaboos University Medical Journal. 6(1) (2006 Jun);33-9. https://pubmed.ncbi.nlm.nih.gov/21748125.
13. Gao D, Zhang Y. Comparative antibacterial activities of extracts of dried ginger and processed ginger. Pharmacognosy Journal. 2(15)(2010):41-4. https://doi.org/10.1016/S0975-3575(10)80077-X
14. Yassen D, Ibrahim AE. Antibacterial activity of crude extracts of ginger (Zingiber officinale Roscoe) on Escherichia coli and Staphylococcus aureus: A Study in vitro. Indo-American Journal of Pharmaceutical Research. 6(06) (2016);5830-35. I SSN NO: 2231-6876
15. Mamede AM, de Souza Coelho CC, Freitas-Silva O, Barboza HT, Soares AG. Lemon. In Nutritional Composition and Antioxidant Properties of Fruits and Vegetables (2020); 377-392. Academic Press.
16. Chung H, Wi S, Cho BK, Lee H. Classification of Garlic (Allium sativum L.) Crops by Fertilizer Differences Using Ground-Based Hyperspectral Imaging System.Agriculture. 14(8) (2024),1215; https://doi.org/10.3390/agriculture14081215
17. Harris JC, Cottrell SL, Plummer S, Lloyd D. Antimicrobial properties of Allium sativum (garlic). Applied microbiology and biotechnology. 57(2001): 282-6.
18. Rana SV, Pal R, Vaiphei K, Sharma SK, Ola RP. Garlic in health and disease. Nutrition research reviews. 24(1) (2011); 60 – 71. DOI: https://doi.org/10.1017/S0954422410000338
19. Martins N, Petropoulos S, Ferreira IC. Chemical composition and bioactive compounds of garlic (Allium sativum L.) as affected by pre-and post-harvest conditions: A review. Food chemistry. 211(15) (2016); 41-50. https://doi.org/10.1016/j.foodchem.2016.05.029
20. Hadi HA, Hasan RH. Effect of plant extracts of Salvia officinalis L. and Costus speciosus L. in growth of some bacteria species isolated from urinary tract infections in Samarra city. Tik. J. of Pure Sci. 25(1) (2020):47-51: http://tjps.tu.edu.iq/index.php/j
21. Yassin MT, Mostafa AA, Al-Askar AA. In vitro, anticandidal potency of Syzygium aromaticum (clove) extracts against vaginal candidiasis. BMC Complementary Medicine and Therapies. 20,25(2020):1-9. https://doi.org/10.1186/s12906-020-2818-8
22. Erhonyota C, Edo GI, Onoharigho FO. Comparison of poison plate and agar well diffusion method determining the antifungal activity of protein fractions. Acta Ecologica Sinica.43(4) (2023):684-689. https://doi.org/10.1016/j.chnaes.2022.08.006
23. Scorzoni L, Sangalli-Leite F, de Lacorte Singulani J, Costa-Orlandi CB, Fusco-Almeida AM, Mendes-Giannini MJ. Searching new antifungals: The use of in vitro and in vivo methods for evaluation of natural compounds. Journal of microbiological methods. 123(2016): 68-78. https://doi.org/10.1016/j.mimet.2016.02.005
24. Peter IU, Edemekong CI, Balogun OM, Ikusika BA, Ngwu J, Ugwueke IV, Ogba RC, Joseph OV, Ogbonna IP, Ubom IJ, Akpu PO. Phytochemical Screening and Antibacterial Activity of Aqueous Extract of Costus afer Plant on Selected Multidrug Resistant

Bacteria. IOSR J Pharmacy and Biol Sciences.17(1) (2022): 39-42.DOI: 10.9790/3008-1701023942

25. Ngemenya MN, Mbah JA, Tane P, Titanji VP. Antibacterial effects of some Cameroonian medicinal plants against common pathogenic bacteria. African Journal of Traditional, Complementary and Alternative Medicines. 3(2), 84–93. Retrieved from https://athmsi.org/journals/index.php/ajtcam/article/view/81
26. Erkmen, O. Practice 18—Antibiotic Sensitivity Test Technique. Laboratory Practices in Microbiology. Academic Press: Cambridge, MA, USA, 2021 Feb 6. 181-186.https://www. Elsevier.com/books and journals.
27. El Moussaoui A, Jawhari FZ, Almehdi AM, Elmsellem H, Benbrahim KF, Bousta D, Bari A. Antibacterial, antifungal and antioxidant activity of total polyphenols of Withania frutescens. L. Bioorganic Chemistry.93 (2019) 103337. https://doi.org/10.1016/j. bioorg.
28. Davis JL, Salmon JH, Papich MG. Pharmacokinetics and tissue distribution of doxycycline after oral administration of single and multiple doses in horses. American Journal of Veterinary Research. 67(2) (2006):310-316. DOI: 10.2460/ajvr.67.2.310
29. Ottou PB, Fogang CW, Ndiang Z, Biyon JB, Mokake SE, Mbezo FB, Betti JL, Ndongo D, Jules R. Assessment of resilience of aromatic plants during the COVID-19 pandemic in Douala, Cameroon. Journal of Drug Delivery and Therapeutics. 12(5) (2022):8-19. DOI: http://dx.doi.org/10.22270/jddt.v12i5.5580
30. Takó M, Kerekes EB, Zambrano C, Kotogán A, Papp T, Krisch J, Vágvölgyi C. Plant phenolics and phenolic-enriched extracts as antimicrobial agents against food-contaminating microorganisms. Antioxidants. 9(2) (2020):165; https://doi.org/10.3390/antiox9020165
31. de Sousa DP, Damasceno RO, Amorati R, Elshabrawy HA, de Castro RD, Bezerra DP, Nunes VR, Gomes RC, Lima TC. Essential oils: Chemistry and pharmacological activities. Biomolecules. 13(7)(2023):1144; https://doi.org/10.3390/biom13071144
32. Sadah Al-Halfi AS, Faisal Al-Shammari MZ, Ibrahim SK. Studying the effect of plant extracts on Gram-positive and Gram-negative pathogens. Journal of University of Anbar for Pure Science.18(01); 76-85(2024).DOI:10.37652/juaps.2023.144339.1159
33. Karuppiah P, Rajaram S. Antibacterial effect of Allium sativum cloves and Zingiber officinale rhizomes against multiple-drug resistant clinical pathogens. Asian Pacific journal of tropical biomedicine. 2(8)(2012):597-601.https://doi.org/10.1016/S2221-1691(12)60104-X
34. Hussein EA, Abd El-Sadek MA, Abd El-Hakem AE, Ismail AM, Aref MS. Pharmacopeial constants, secondary metabolites and antibacterial activity of calli induced in vitro from flaxseed sprouts explants. International Journal of Theoretical and Applied Research. 2(2) (2023):202-8. DOI: 10.21608/ijtar.2023.202633.1047
35. Abd El Latif, H. K., Ibrahim, R. H., El-Ganiny, A. M., & Abd El Sabour, H. I. (2014). Study on biofilm formation by Extended Spectrum β-lactamase (ESBL) producing Klebsiella isolates. Zagazig Journal of Pharmaceutical Sciences, 23(2) (2014); 66-74.DOI: 10.21608/zjps.2014.38186
36. Salisu A, Iliyasu MY, Sahal MR, Titus I, Isma'il S, Umar RD, Tahir H, Kabeer ZM, Idris AH, Musa HS, Agbo EB. Antimicrobial potential of *Syzygium aromaticium* (clove) extracts on multidrug-resistant (MDR) uropathogenic bacteria isolated from clinical specimens in Bauchi, Nigeria. Methodology. *Journal of Advances in Medical and Pharmaceutical Sciences*. 24(11-12) (2022):41-57, DOI: 10.9734/JAMPS/2022/v24i11588
37. Vázquez-Ucha JC, Martínez-Guitián M, Lasarte-Monterrubio C, Conde-Pérez K, Arca-Suárez J, Álvarez-Fraga L, Pérez A, Crecente-Campo J, Alonso MJ, Bou G, Poza M. Syzygium aromaticum (clove) and Thymus zygis (thyme) essential oils increase susceptibility to colistin in the nosocomial pathogens Acinetobacter baumannii and Klebsiella pneumoniae. Biomedicine & Pharmacotherapy.130(2022) 110606. https://doi.org/10.1016/j.biopha.2020.110606
38. Tshabalala R, Kabelinde A, Tchatchouang CD, Ateba CN, Manganyi MC. Effect of Clove (Syzygium aromaticum) spice as a microbial inhibitor of resistant bacteria and Organoleptic Quality of meat. Saudi Journal of Biological Sciences. 28(7) (2021):3855-3863.https://doi.org/10.1016/j.sjbs.2021.03.052
39. Ceylane A, Daniel Y. Antimicrobial activity of spices. Journal of rapid methods and automation in Microbiology. 12(1) (2004):1-55. https://doi.org/10.1111/j.1745-4581.2004.tb00046.
40. Iotsor BI, Iseghohi F, Oladoja OE, Raji OR, Yusuf Z, Oyewole OA. Antimicrobial activities of garlic and ginger extracts on some clinical isolates. Int. J. Biotechnol. 8(1) (2019) 59-65. DOI:10.18488/journal.57.2019.81.59.65
41. Banik A, Abony M, Zerin T, Datta S. Antibacterial activity of Allium sativum, Syzygium aromaticum, and Cinnamomum zeylanicum against foodborne pathogens in vitro. IOSR Journal of Pharmacy and Biological Sciences. 13(2018): 68-73. DOI:10.9790/3008-1302056873
42. El-Maati MF, Mahgoub SA, Labib SM, Al-Gaby AM, Ramadan MF. Phenolic extracts of clove (Syzygium aromaticum) with novel antioxidant and antibacterial activities. European journal of integrative medicine. 8(4) (2016):494-504. DOI:10.1016/j.eujim.2016.02.006
43. Mir IH, Sharma MK, Singh A. Antimicrobial activity of some medicinal plant extracts against multidrug-resistant bacteria. Journal of Advanced Scientific Research. 2021 Mar 31;12(01 Suppl 1):42-7.
44. Arora DS, Kaur J. Antimicrobial activity of spices. International journal of antimicrobial agents. 12(3) (1999):257-262.

https://doi.org/10.1016/S0924-8579(99)00074-6
45. Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li HB. Antibacterial and antifungal activities of spices. International journal of molecular sciences. 18(6) (2017):1283. https://doi.org/10.3390/ijms18061283
46. Sethi S, Dutta A, Gupta BL, Gupta S. Antimicrobial activity of spices against isolated food borne pathogens. International Journal of Pharmacy and Pharmaceutical Sciences. 5(1) (2013):260-262.
47. Banik A, Abony M, Zerin T, Datta S. Antibacterial activity of Allium sativum, Syzygium aromaticum, and Cinnamomum zeylanicum against food borne pathogens in vitro. IOSR Journal of Pharmacy and Biological Sciences;13(2) (2018): 68-73. DOI: 10.9790/3008-1302056873
48. Balamurugan J, Kumar TT, Prakash S, Meenakumari B, Balasundaram C, Harikrishnan R. Clove extract: A potential source for stress free transport of fish. Aquaculture. 454(2016): 171-175. https://doi.org/10.1016/j.aquaculture.2015.12.020
49. Arniati A, Pratama M, Baits M, Tahir M. Determination of Total Phenolic Levels of Ethyl Acetate Fraction of Clove Leaves (Syzygium aromaticum (L) Merr). Pharmaceutical Reports. 2022 Oct 8;1(2).DOI: https://doi.org/10.33096/pharmrep.v1i2.167
50. Karuppiah P, Rajaram S. Antibacterial effect of *Allium sativum* cloves and *Zingiber officinale* rhizomes against multiple-drug resistant clinical pathogens. Asian Pacific Journal of Tropical Biomedicine. 2(8) (2012); 597-601. https://doi.org/10.1016/S2221-1691(12)60104-X
51. Malu SP, Obochi GO, Tawo EN, Nyong BE. Antibacterial activity and medicinal properties of ginger (Zingiber officinale). Global Journal of pure and applied Sciences.; 15(3-4) (2009). DOI:10.4314/gjpas. v15i3-4.48561
52. Singh AA, Naaz ZT, Rakaseta E, Perera M, Singh V, Cheung W, Mani F, Nath S. Antimicrobial activity of selected plant extracts against common food borne pathogenic bacteria Food and Humanity 1(2023); 64-70. https://doi.org/10.1016/j.foohum.2023.04.002
53. Afanyibo YG, Anani K, Esseh K, Sadji Y, Idoh K, Koudouvo K, Agbonon A, Améyapoh Y, Tozo K, Gbeassor M. Antimicrobial activities of Syzygium aromaticum (L.) Merr. & LM Perry (myrtaceae) fruit extracts on six standard microorganisms and their clinical counterpart. Open Access Library Journal. 5(12) (2018):1-3. Doi: 10.4236/oalib.1104951
54. Behbahani BA, Noshad M, Falah F. Study of chemical structure, antimicrobial, cytotoxic and mechanism of action of Syzygium aromaticum essential oil on foodborne pathogens. Slovak Journal of Food Sciences/Potravinarstvo. 13(1) (2019). https://doi.org/10.5219/1226
55. Bikheet MM, Hassan HM, Omar MO, Abdel-Aleem WM, Galal SM, Korma SA, Ibrahim SA, Nassar KS. Effects of clove (Syzygium aromaticum) extract on antibacterial activity, phytochemical properties, and storage quality of flavored milk beverages. Journal of Dairy Science.108(4) (2025):3300–3313. https://doi.org/10.3168/jds.2024-26023
56. Zeinab A ES, Aisha M AA. ANTI-TUMOR ACTIVITIES AND OIL CONSTITUENTS OF ZINGIBER OFFICINALE (ZINGIBERACEAE. Arab Universities Journal of Agricultural Sciences. 19(1)(2021):153-159. https://doi: 10.21608/ajs.2011.14642
57. El-Desoukey RMA, Albarakaty FM, Alqahtani NM, Lina AL, Safaa AA, et al. Unexpected Antimicrobials. Journal of Plant Biology and Crop Research. 5(1) (2022): 1049.Online; http://meddocsonline.org/
58. Li H, Li J, Hua Z, Aziz T, Khojah E, Cui H, Lin L. Transcriptomic combined with molecular dynamics simulation analysis of the inhibitory mechanism of clove essential oil against Staphylococcus aureus biofilm and its application on surface of food contact materials. Food and Bioproducts Processing. 150(2025); 131-140 https://doi.org/10.1016/j.fbp.2025.01.009
59. Bai J, Li J, Chen Z, Bai X, Yang Z, Wang Z, Yang Y. Antibacterial activity and mechanism of clove essential oil against foodborne pathogens. Lwt. 173(2023): 114249. https://doi.org/10.1016/j.lwt.2022.114249
60. Aldabaan NA, Turakani B, Mahnashi MH, Shaikh IA, Alhazmi AY, Almasoudi HH, Abdulaziz O, Khuwaja G, Khan AA, Basavegowda N, Dafalla SE. Evaluation of antimicrobial, anticancer, antidiabetic, antioxidant activities and silver nanoparticles synthesized from Indian Clove-Syzygium aromaticum leaf extract. Journal of King Saud University-Science. 36(4) (2024):103142. https://doi.org/10.1016/j.jksus.2024.103142
61. Dhanapal R, Parasuraman S, Nizami MZ, Lin GS, Dutta K. Ginger's bite: A systematic review on the effectiveness of Zingiber officinale in reducing dental caries. Intelligent Pharmacy. 2(5)2024; 638-642. https://doi.org/10.1016/j.ipha.2024.08.001
62. Sethunga M, Ranasinghe MM, Ranaweera KK, Munaweera I, Gunathilake KD. Synergistic antimicrobial activity of essential oils and oleoresins of cinnamon (Cinnamomum zeylanicum), clove bud (Syzygium aromaticum) and ginger (Zingiber officinale). Biocatalysis and Agricultural Biotechnology. 51(2023): 102800. https://doi.org/10.1016/j.bcab.2023.102800
63. Akullo JO, Kiage B, Nakimbugwe D, Kinyuru J. Effect of aqueous and organic solvent extraction on in-vitro antimicrobial activity of two varieties of fresh ginger (Zingiber officinale) and garlic (Allium sativum). Heliyon. 8(9) (2022). http://dx.doi.org/10.1016/j.heliyon.2022.e10457
64. Otang WM, Afolayan AJ. Antimicrobial and antioxidant efficacy of Citrus limon L. peel extracts used for skin diseases by Xhosa tribe of Amathole District, Eastern Cape, South Africa. South African Journal of Botany. 102(2016): 46-9. https://doi.org/10.1016/j.sajb.2015.08.005